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Progress in Anaesthesia

Twelve years ago the writer of the preface to a symposium on anaesthesia and analgesia (*Brit. med. Bull.* 1946, Vol. 4, No. 2) expressed the belief that future developments in this field of medicine would provide increasing scope for collective scientific work by the chemist, physiologist, pharmacologist and anaesthetist, with even the physicist playing a part in the solving of special problems. That this prediction would be fulfilled was fairly clear because, at the time it was made, the complexities of new anaesthetic techniques were already demanding much more science than art from anaesthetists. There was indeed an urgent need of expert aid and knowledge whereby newly perceived physical, biochemical and pharmacological problems involving gross disturbances of physiological function in anaesthetized patients could be investigated experimentally and clinically under properly controlled conditions.

A number of articles in the present symposium, though not of joint authorship, reflect the growth of collaboration in the past decade between anaesthetists and workers in the basic medical sciences and in physics. The association of anaesthetists with physicists has been particularly welcome and profitable because, in the first instance, it dealt successfully with a

long-standing omission to define and establish certain standards in design and construction, such as might ensure that anaesthetic equipment and the way it was used would comply with physiological requirements. Secondly, as anaesthetists began to extend their interests and activities beyond surgical units and to assist in the care of patients suffering from respiratory paralysis or inadequacy from various causes, close co-operation with physicists became even more essential and has led to the successful development of several types of mechanical respirators.

Dealing every day with the problem of promoting effective pulmonary ventilation in patients under the influence of medullary depressants and muscle relaxants, most anaesthetists can advise on the management of the respiratory difficulties so frequently associated with poliomyelitis, tetanus and narcotic poisoning. In addition, their familiarity with respirators which, incidentally, may be readily acquired by employing such apparatus in place of conventional anaesthetic machines, together with competence in bronchoscopic aspiration of pulmonary secretions, enables anaesthetists to provide the special technical aid that is indispensable if the treatment of respiratory inadequacy is to be effective. This particular skill is now being more widely applied to prevent or relieve asphyxia in cases of pulmonary oedema resulting from cardiac failure or chronic respiratory disease. Service of this kind can be life-saving, albeit only temporarily, in patients who for lack of such active treatment may be prematurely abandoned.

In the years that have elapsed since the previous symposium, innovations in anaesthetic practice have been numerous. How many of them merit the title of "advances" in the same way that endotracheal intubation, controlled respiration and the introduction of specific muscle relaxants did, is doubtful. One or two have been substantial enough to survive despite strong divergence of views, so in all fairness, judgement, in respect to them, must still wait.

Among the sixteen papers which follow this introduction are two on induced hypotension. Experimental and clinical investigations have now largely caught up with and modified the impressionism that characterized many of the earlier presentations concerning this subject, and the present antagonists now discuss this ancillary procedure dispassionately from fundamental data. Whatever its final evaluation as an adjunct to anaesthesia there is no doubt that this controversial technique has stimulated studies which have added considerably to our knowledge of circulatory function.

Like induced hypotension, the deliberate production of hypothermia has raised many problems in biochemistry and haemodynamics. Here again there has been a lag in the experimental work in relation to the liberality with which some clinicians have applied it in a wide variety of circumstances, not all of which would appear to be justifiable or likely to be ratified in the future. While the range of application of hypothermia has not yet been determined, its use during cardiac surgery has meantime provided a unique opportunity for a systematic study of the mechanisms of cardiac arrest and ventricular fibrillation and their appropriate treatment under what have virtually been experimental conditions.

Anaesthetists will appreciate the combined scientific and practical value of the several papers presented by scientist contributors who have devoted a considerable amount of time in recent years to fundamental studies closely related to some of the clinical problems of anaesthesia. For those of us whose abilities and experience are confined to clinical work the inclusion of "slabs" of basic science is doubtless salutary even if we only sit and stare for a little at incomprehensible equations and look in vain for the familiar x and y of our youth.

There is, however, much to restore the equanimity of the non-scientific reader in the reports of purely clinical investigations. Even in those it is gratifying to note that the influence of the scientists can be seen in the character and critical standards of the papers presented. Another remarkable feature is that the clinical articles are distinguished as much by the competent discussion by anaesthetists of surgical, physiological and pharmacological factors as by the presentation of the anaesthetic procedure—a commendable trend which enhances the value of such contributions.

Although it has not been considered in the symposium, probably the greatest change and forward step has concerned the training and status of those who practise in this specialty. Much that was haphazard, unorganized and most appropriately described as "learning the hard way" has been, or is in process of being, eliminated. For the most beneficial application of new anaesthetic procedures which to some extent had outstripped the basic scientific knowledge of many who were expected to use them, it became imperative to set up rigid criteria in respect to training. The establishment of a central academic body, the Faculty of Anaesthetists, within the Royal College of Surgeons of England, and of a higher diploma of Fellowship, has imposed on candidates for permanent staff appointments in the hospital service a pattern of study, preparation and examination similar to that for consultants in other branches of medicine. The high standard set from the start in both the Primary and Final parts of the examination has assured respect for it, and encouraged the best quality of entrant to the specialty. Beyond that, the latent beneficent effects of an academic discipline must not be forgotten. The study of the basic medical sciences for the two years or thereby, which the average candidate appears to need, should endow the young graduate with a fundamental outlook and a sound critical faculty in respect to his own work and that of others. On this foundation he will be able through ever-growing experience to build up the skill and knowledge essential to meet the challenging demands of his surgical associates as they probe the innermost recesses of the human body, and in this and other less dramatic aspects of his work continue to serve mankind well.

John Gillies

We are very grateful to Professor J. H. Burn and Dr Ronald Woolmer for all their help and advice as Chairman of the planning committee, and Scientific Editor, respectively, of this symposium.

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DR J. SELWYN CRAWFORD, after qualifying M.B., Ch.B. (Leeds) in 1947, undertook the usual early postgraduate hospital appointments, followed by two years (1950–52) as an anaesthetist in the Royal Air Force. He obtained the Diploma in Anaesthetics (London) in 1952, and was elected to the Fellowship of the Faculty of Anaesthetists, Royal College of Surgeons of England, in 1954. After holding appointments in anaesthesia at Leicester Royal Infirmary and the Central Middlesex Hospital, London, he went in 1955 to work as an anaesthetist in Salisbury, Southern Rhodesia, returning to Europe the following year. For a subsequent six months, he held an appointment at Ullevål Hospital, Oslo, Norway, from where he moved to Portsmouth, England, for a year. In October 1957, Dr Selwyn Crawford took up the post of Visiting Instructor Anaesthetist to the University of Pittsburgh at the Mercy Hospital in that city. He has published a paper entitled: "Some aspects of obstetric anaesthesia" (*Brit. J. Anaesth.* 1956, **28**, 146, 201).

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DR H. G. EPSTEIN is First Assistant to the Nuffield Department of Anaesthetics, University of Oxford. He was originally trained as a physico-chemist and made contributions to the fields of chemical reactions, low-temperature spectroscopy and ultrasonics before the Second World War. Since 1940 he has been applying various physical methods to anaesthetic research, and has also worked on allied problems in the Departments of Pharmacology and Physiology. He has been actively engaged on the design of anaesthetic appliances and analgesic inhalers. During the past decade he has been a member of various committees of the Medical Research Council, the last on non-explosive anaesthetics. He is a graduate member of the University of Oxford and a Fellow of the Faculty of Anaesthetists, Royal College of Surgeons of England. Dr Epstein collaborated in a book on chemical gas reactions published in America in 1943, and is co-author with Sir Robert Macintosh and Professor W. W. Mushin of *Physics for the anaesthetist* (2nd ed., in press). Several other publications deal with new anaesthetic apparatus, e.g., *Anaesthesia*, 1956, **11**, 83. In recent years he has worked on respiration recorders, on the resistance of anaesthetic appliances, and on quantitative inhalers for Fluothane.

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Dr G. Jackson Rees, a graduate of the University of Liverpool, began to practise anaesthetics in the Royal Air Force, and in 1947 became the first demonstrator in the newly formed Department of Anaesthesia at the University of Liverpool. He has been responsible for the development of paediatric anaesthesia in his home city and is in charge of the anaesthetic service of the Neonatal Unit at Alder Hey Children's Hospital and the Cardiac Unit at the Royal Liverpool Children's Hospital. He also holds the post of Lecturer in Paediatric Anaesthesia at the University of Liverpool.

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Professor D. Whitteridge is Head of the Department of Physiology, University of Edinburgh, and was previously Fellow of Magdalen College and University Demonstrator at Oxford. He has worked on the nature of small nerve-fibres arising from the heart and lungs, on the physiology of spinal transection in man and on the proprioceptors from eye muscles and their central connexions. This has led on to work on the central projections from the retina and the associated postural mechanisms. His Presidential Address to Section I of the British Association was on "The machinery of posture" (*Advanc. Sci., Lond.* 1956, **13**, 104). He was elected a Fellow of the Royal Society in 1953.

Dr L. J. Wolfson was Anaesthetist to the Lewisham General Hospital from 1938 to 1941. Since 1941 he has been Senior Consultant Anaesthetist at the Birmingham Accident Hospital (except for two years spent as Specialist Anaesthetist in the Royal Army Medical Corps). From 1933 to 1938 he did hospital work in anaesthesia, general medicine, surgery and pathology. Since then he has been engaged solely with anaesthesia. His published work includes: "Anaesthesia in traumatic shock" (*Brit. J. clin. Pract.* 1956, **10**, 783); and "The treatment of acute poisoning" (*J. industr. Nurses*, 1956, **8**, 75). Dr Wolfson is the President of the Anaesthetic Section of the Birmingham Medical Institute for 1957–58.

Dr Ronald Woolmer is Director of the recently established Research Department of Anaesthetics in the Royal College of Surgeons of England. He is also Consultant in Anaesthetics to the Royal Navy and Honorary Consultant Anaesthetist to the Whittington Hospital, London. Until recently he was Reader in Anaesthetics in the University of Bristol. His published works include papers on various aspects of anaesthesia.

Dr Barry Wyke graduated from the University of Sydney, where he was Lecturer in the Anatomy and Physiology of the Nervous System, and Liston Wilson Research Fellow in Neurosurgery. Following election to a Rockefeller Foundation Fellowship he worked in several neurosurgical and neurophysiological centres in the USA. Dr Wyke then came to Oxford to join Sir Hugh Cairns' Neurosurgical Unit at the Radcliffe Infirmary: while there he was elected to a Beit Memorial Research Fellowship. At present he is Senior Lecturer in Applied Physiology at the Royal College of Surgeons of England, and Honorary Clinical Assistant in Neurological Surgery at the Brook Hospital, Woolwich, in charge of the Neurosurgical Research Unit. His principal clinical and experimental work deals with the cortical control of movement in relation to the diagnosis and surgical treatment of seizures, as a result of which he was elected by the Royal College of Surgeons to an Arris and Gale Lectureship in 1954, and to an Arnott Demonstratorship in 1957. It was in the course of this work that he developed the technique of electrographic monitoring of anaesthesia, which is described in a series of papers in *Anaesthesia* and in this Bulletin. His other publications include a chapter on "Biophysical aspects of nervous function" in *Progress in Biophysics and Biophysical Chemistry* (1951), and "Surgical considerations of the temporal lobes" in the *Annals of the Royal College of Surgeons of England* (1957).

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EFFECT OF ANAESTHETICS ON MECHANICAL RECEPTORS

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1 Pulmonary stretch receptors
2 Carotid sinus receptors
3 Other mechanical receptors
4 Pulmonary deflation endings
5 Discussion
a Mode of action
b Reflex effects
References

Although the primary point of attack of general anaesthetics is within the central nervous system, we frequently have also to consider their direct effects on muscle and the neuromuscular junction, and on autonomic ganglia. Though conduction in nerve trunks is known to be unaffected by anaesthetics in usual concentrations, the terminal nerve fibres or the receptor apparatus may be more susceptible. Here drugs may produce stimulation, sensitization or block.

The distinction between stimulation and sensitization has recently been stressed by Paintal (1956). A substance may sensitize a nerve-ending to its physiological stimulus, or it may stimulate the ending in the absence of a physiological stimulus. If frequency of response is plotted against strength of stimulus, a chemical substance may be seen to sensitize an ending, so increasing the slope of the curve (fig. 1). Alternatively it may stimulate and desensitize, so making the curve more nearly horizontal. It may also affect the threshold of the ending, so moving the curve bodily up or down (fig. 2).

1. Pulmonary Stretch Receptors

Thus, as Paintal (1956) points out, volatile anaesthetics sensitize pulmonary stretch receptors to inflation (Whitteridge & Bülbring, 1944) without much change in their threshold, whereas the veratrine alkaloids stimulate and simultaneously desensitize pulmonary stretch receptors (Dawes, Mott & Widdicombe, 1951; Meier, Bein & Helmich, 1949).

A wide variety of volatile anaesthetics sensitize afferent endings, as do a number of substances too toxic to be used as anaesthetics. The non-volatile anaesthetics, urethane and the barbiturates, not only do not excite nerve-endings, but may, in sufficiently high doses, depress them.

Pulmonary stretch endings are perhaps the easiest to study experimentally, and all volatile anaesthetics tested produce a sensitization which raises the peak frequency of response by 100% or more, depending on dosage and the range of stimuli used.

Chloroform, ethyl chloride, trichloroethylene, ethyl ether, divinyl ether—all have rather similar effects. Doses normally used for maintenance of anaesthesia stimulate and may not paralyse, but doses reached during induction of anaesthesia usually paralyse the nerve-endings (Whitteridge & Bülbring, 1944). These observations have been confirmed by Dawes, Mott & Widdicombe (1951) and Paintal (1957). The anaesthetic gases cyclopropane and nitrous oxide both sensitize pulmonary stretch receptors, but do not paralyse them even in concentrations of 50% cyclopropane and 100% nitrous oxide. As this sensitization may continue unchanged for many minutes, these gases seem particularly appropriate for investigation of the mechanism of sensitization. Some new work by Lundy & Whitteridge (unpublished) has confirmed the earlier results on nitrous oxide, and has shown that even 50% nitrous oxide produces considerable sensitization.

In some preliminary experiments with Fluothane[1], sensitization of pulmonary stretch receptors with some rise in threshold was seen, when concentrations likely to be reached in the early stages of anaesthesia were used. Lundy and Whitteridge have also confirmed that carbon tetrachloride acts similarly to chloroform, and that acetone and ethanol also sensitize these endings to a certain extent.

[1] Halothane has recently been adopted by the British Pharmacopoeia Commission as the approved name for this substance.—Ed.

FIG. 1. SENSITIZATION OF A PULMONARY STRETCH ENDING BY INFLATION WITH 50% CYCLOPROPANE

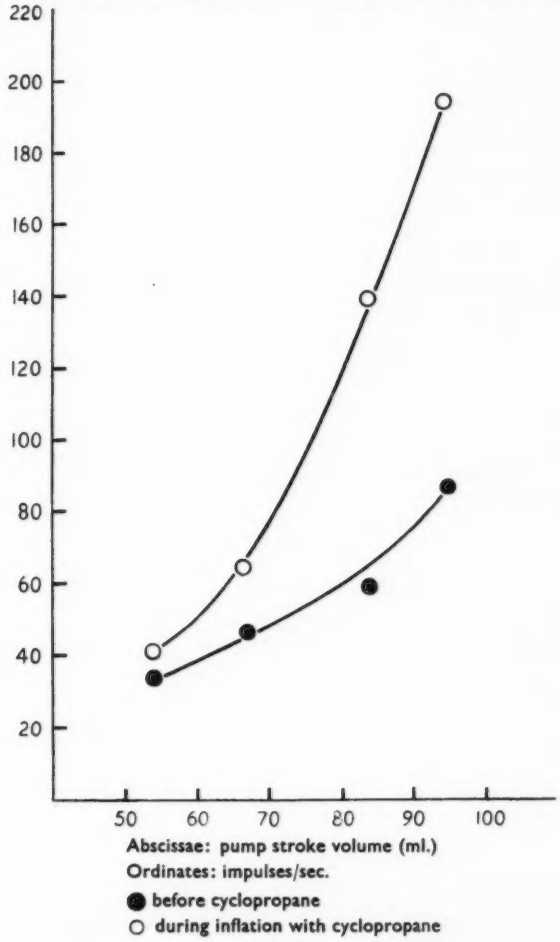
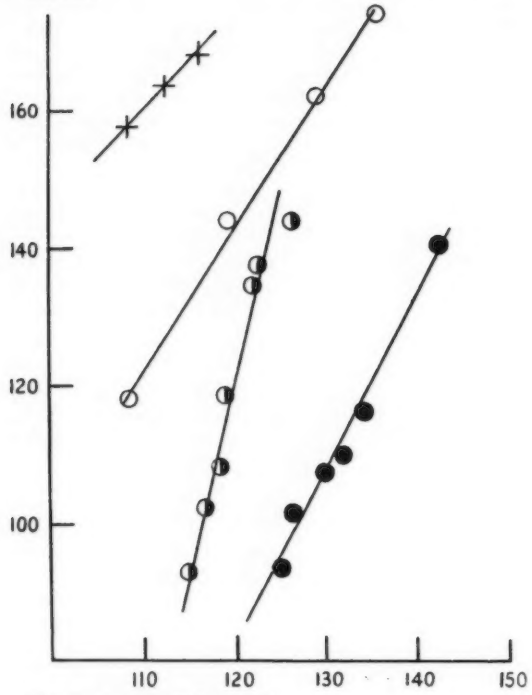


FIG. 2. SENSITIZATION OF A PRESSURE RECEPTOR FROM THE CAROTID SINUS, FOLLOWED BY DESENSITIZATION BY INHALATION OF 20% ETHER



Abscissae: pressure (mm. Hg)

Ordinates: impulses/sec.

● control observation

◑ after administration of 20% ether for 30 sec.

○ after administration of the same concentration of ether for 1.5 min.

\+ after administration of the same concentration of ether for 4.5 min.

At first the nerve-ending is sensitized, but although its frequency of discharge remains high, after 1 min. it becomes less sensitive to a change in carotid blood pressure.

2. Carotid Sinus Receptors

A similar sensitization of carotid sinus and aortic pressure receptors has been observed by Robertson, Swan & Whitteridge (1956). These endings are sensitized by trichloroethylene, chloroform, and ether, and may also show considerable changes in threshold. These effects are present in the perfused carotid sinus, where they cannot be due to changes in the amount of circulating adrenaline acting on the artery wall.

No effect whatever of nitrous oxide nor of cyclopropane could be observed on these endings.

3. Other Mechanical Receptors

Matthews (1933) described a similar effect of ether on muscle spindles in the cat. The muscle spindle offers a possible preparation on which to analyse the mechanism.

4. Pulmonary Deflation Endings

The existence of pulmonary endings sensitive to deflation had been inferred by Head (1889), Hammouda & Wilson (1935) and by Whitteridge & Bülbring (1944), in each case on evidence of the persistence of deflation reflexes after some selective blocking agent had abolished the inspiratory component of the Hering-Breuer reflex. The main effect of the deflation reflex is a maintained contraction of the diaphragm, but there may also be an increase in the respiratory rate. During anaesthesia with trichloroethylene there is a contraction of the diaphragm which persists throughout expiration and is accentuated in inspiration. It is accompanied by, and presumably causes, an increase in functional residual capacity which is not produced by any other anaesthetic agent tried (Whitteridge & Bülbring, 1944). In their comprehensive review, Dawes & Comroe (1954) described a pulmonary respiratory chemo-reflex with a number of possible components; the deflation reflex; the vascular reflex of multiple minute embolism; the high-threshold inhibitory reflex of Widdicombe (1954); and the pulmonary respiratory chemoreflex. Only the first unequivocally increases expiratory activity of the diaphragm. Subsequently Paintal (1955, 1957) has described " true " deflation fibres of very small diameter, excited by sudden deflation and by phenyldiguanide. Some of these fibres are sensitized and later paralysed by trichloroethylene and by ether (Paintal, 1957). It is not yet clear whether these deflation receptors are responsible for the increased diaphragmatic activity which is characteristic of the deflation reflex. Unfortunately the effect of the phenyldiguanide is probably to excite a wide variety of very small fibres. According to Paintal (1957) the deflation fibres arise from the respiratory bronchioles and atria.

5. Discussion

a. Mode of Action

If one assumes the existence of a generator potential set up by the physical stimulus and capable of exciting the terminal nerve fibre, there are several theoretical points of action of anaesthetics: (i) they might increase the amplitude of the generator potential set up by a given physical stimulus; (ii) they might so change the electrical properties of the cell membrane of the nerve fibre as to make a constant generator potential a more effective stimulus to the nerve; (iii) they might depolarize the terminal nerve fibre so that a constant generator potential becomes a more effective stimulus; (iv) they might increase the ability of the nerve fibre to set up a repetitive train of impulses.

There is direct evidence for (iii) from the work of Berney & Posternak (1956), who find that the lower aliphatic alcohols, chloroform, ether and acetone depolarize myelinated fibres in the frog's sciatic nerve, and also lower their threshold to stimulation. Urethane and the higher aliphatic alcohols hyperpolarize the nerve, and block without stimulating. The depolarizing effect and anaesthetic potency do not run parallel (Posternak & Zahnd, 1953).

In some very elegant experiments, Arvanitaki & Chalazonitis (1951) showed that ether and chloroform increased the excitability of the isolated giant fibre of *Sepia* for about 10 seconds—" surexcitabilité prénarcotique ". After withdrawal of the anaesthetic this increased excitability reappeared—" surexcitabilité métanarcotique ". In the earliest stages ether and chloroform depolarize the nerve fibre and

probably increase the rectification at cathode and anode. When narcosis is established, the rectification effect at anode and cathode tends to decrease. Fibres which were made hyperexcitable by withdrawal of calcium showed rapid repetitive discharge during the phase of "surexcitabilité prénarcotique" when stimulated by currents normally sub-threshold.

On the other hand, urethane and chloral never produced phases of increased excitability. This behaviour seems so closely parallel to the observations on nerve-endings as to suggest that this is the most likely explanation yet available.

b. Reflex Effects

Any practical importance this phenomenon has depends on its reflex effects, not on its mode of origin. The original evidence that shallow breathing—a reduction in tidal volume—may be due to sensitization of pulmonary stretch endings is reasonably satisfactory (Whitteridge & Bülbring, 1944). However, the reduction in tidal volume with cyclopropane seems to be slight, and with ether and trichloroethylene, which may reduce the tidal volume by 50%, the initial effect is certainly transitory. The suggestion that there may be some sensitization of deflation receptors has received support from Paintal (1957) who has obtained records from such receptors and observed their sensitization. If it were possible to eliminate this effect, the usefulness of trichloroethylene in deep anaesthesia would be very much greater.

The sensitization of pressure receptors in the carotid sinus probably also plays an important part in anaesthesia. Robertson & Swan (1957) added ether and chloroform to the fluid perfusing an isolated carotid sinus in the cat. In each case the effect of an increase in perfusion pressure on the heart rate and blood pressure was enhanced. Although volatile anaesthetics may stimulate chemo-receptors, they would not thereby produce a fall in heart rate or blood pressure. It is possible that cardiac inhibition produced by chloroform in the absence of atropine is due, at least in part, to the same mechanism. On the other hand, after section of all four moderator nerves, a small drop in blood pressure can still be brought about by trichloroethylene. Some preliminary work with Fluothane suggests that this substance has little if any effect on carotid sinus receptors even in doses which produce a sharp fall in blood pressure.

There is, of course, no reason to expect that the stimulating effect of drugs on receptors will run parallel to their anaesthetic potency, which is dependent on their effect on cells and synapses in the central nervous system (cf. Dripps & Severinghaus, 1955). They must be acting on the terminal nerve fibre or the receptor, structures which are remarkably resistant to anoxia and in this point chemically unlike the central synapses. In addition the local concentration of anaesthetic agent at receptors and in the central nervous system will seldom be the same. In the closely similar effect of anaesthetics on peripheral nerve fibres, increasing the molecular weight in the aliphatic series increased the anaesthetic potency but decreased the lowering of theshold (Posternak & Zahnd, 1953).

This curious effect of an increase in excitability at the onset of anaesthetic action seems to be peculiar to the members of lower molecular weight in homologous series with anaesthetic effects. It is most readily explained as an effect on the cell membrane, perhaps primarily increasing permeability at the cathode and decreasing it at the anode, and accompanied by some degree of depolarization and increase in excitability. Clearly it needs analysis in terms of ion transport. It seems to be an effect quite distinct from narcosis.

* * *

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BIOCHEMICAL DISTURBANCES ASSOCIATED WITH ANAESTHESIA

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Since Cuthbertson (1930) described an increase in urinary nitrogen output with a negative nitrogen balance following injury, there have been numerous reports on the metabolic response of patients to anaesthesia and surgical operation (Wilkinson, Billing, Nagy & Stewart, 1949, 1950a, 1950b, 1951; Moore & Ball, 1952; Le Quesne, 1957; Wilkinson, 1955). It is now evident that post-traumatic protein catabolism is only part of a wide-spread disturbance involving water and electrolyte balance, renal and hepatic function and general cell metabolism. These changes are thought to be due partly to cellular damage and partly to altered endocrine activity, especially of the adrenal cortex (Stewart, 1955).

In most of the reports referred to, it is difficult to assess the contribution of the anaesthetic to post-operative disturbances of metabolism, for, in addition to trauma, other modifying factors, such as pre-operative starvation, concurrent disease, variations in blood pressure and gaseous exchange during operation as well as post-operative complications and treatment, are often present. Proper evaluation of the effects of anaesthetic drugs and procedures can be made only on anaesthetized persons or animals who are not to be subjected to surgery, or after the induction of anaesthesia but before surgery is begun. It must, however, be remembered that any biochemical disturbances produced by anaesthesia may be modified by the operation and by other factors already mentioned. With this in mind we shall review briefly the biochemical disturbances which follow anaesthesia (including the effects of adjuvants such as the muscle-relaxant drugs, controlled hypotension and hypothermia), and the possible mechanisms concerned in their production. We shall, however, exclude so far as we can those metabolic changes which are attributable to surgery. Consideration of the older and more toxic drugs formerly given alone to produce anaesthesia for long and extensive operations has for the most part been omitted, as also have studies dealing primarily with the mode of action and with the breakdown and excretion of anaesthetic drugs.

1. Acid-Base Imbalance in Anaesthesia

a. Imbalance of Respiratory Origin

Elimination of carbon dioxide by the lungs is quantitatively the main mechanism for the excretion of the acidic end-products of metabolism; the others, secretion of acid urine and excretion of anions as ammonium salts, are by comparison slow. While theoretically the plasma buffers can take up the carbon dioxide produced in about five minutes, their role is purely temporary. When breathing is inadequate through depression or obstruction, carbon-dioxide retention occurs and the plasma pH ultimately falls as a result of a decrease in the bicarbonate:carbonic acid ratio. Conversely, when ventilation is excessive, respiratory alkalosis occurs with reduced carbon-dioxide tension in the plasma and an increase in the bicarbonate:carbonic acid ratio.

Such deviations from balance tend to be self-limiting where respiratory reflex control is active. Current anaesthetic procedures interfere with these reflexes, both centrally and peripherally, and place on the anaesthetist the onus of ensuring adequate oxygenation and carbon-dioxide elimination. Thus excessive pulmonary ventilation can reduce the arterial carbon-dioxide tension to a level such as may be reached in the conscious subject only after vigorous hyperventilation (Woolmer, 1956). On the other hand, where a gas mixture containing a high percentage of oxygen is used, the tidal exchange, whether respiration is spontaneous or assisted, may be adequate to maintain full oxygenation but insufficient to prevent retention of carbon dioxide. This situation is especially common where ineffective spontaneous breathing is allowed to persist in patients who have been given muscle relaxants, who are anaesthetized by closed-circuit techniques or who are undergoing thoracotomy (Dripps, 1947; Beecher & Murphy, 1950; Taylor & Roos, 1950; Maier, Rich & Eichen, 1951; Etsten, 1953; Stead, Martin & Jensen, 1953; Pask, 1955).

Lawes (1956) quotes several authors to show that the signs of hypercapnia are very variable and depend not only upon the degree of retention but also upon the susceptibility of the individual patient to carbon dioxide. He concludes that instrumental aid is necessary during anaesthesia to assess the levels of carbon dioxide in blood.

Hypothermia may cause respiratory acidosis because of a reduction in the rate and volume of respiration and the increased solubility of carbon dioxide in the plasma at low body-temperatures (Rosenhain & Penrod, 1951). Although the carbon-dioxide content of the blood is increased during hypothermia this does not necessarily lower the plasma pH (Brewin, Nashat & Neil, 1956). It has been claimed, however, that during hypothermia a low plasma pH due to carbon-dioxide retention is an important factor in the causation of ventricular fibrillation and that the maintenance of hyperventilation reduces the incidence of this serious complication (Osborn, 1953; Swan, Zeavin, Holmes & Montgomery, 1953; Fleming, 1954; Boeré, 1956a, 1956b).

Foldes (1957) believes that acidosis may be an important factor in slowing the spontaneous hydrolysis of suxamethonium and may be one cause of the prolonged apnoea which occasionally follows the use of this agent.

Sudden reversal of the hypercapnic state may lead to hypotension (Dripps, 1947) or even to cardiac arrest (Miller, Brown, Buckley, Van Bergen & Varco, 1952). Lasting effects of respiratory acidosis on general metabolism are probably slight and tend to be obscured by the metabolic effects of operative stress.

Long-term effects of respiratory alkalosis are similarly ill-defined and are probably not serious. Post-operative tetany is rare, and the possible influence of respiratory alkalosis on the production of renal calculi is not a problem in patients anaesthetized for surgical procedures, though it may be of some importance in patients with poliomyelitis who are subjected to prolonged hyperventilation (Lassen, 1955).

b. Imbalance of Metabolic Origin

Anaesthetic agents acting indirectly on cellular processes may themselves cause acidosis of metabolic type. In particular, deep ether anaesthesia is associated with slightly increased plasma concentrations of metabolic anions, such as lactate and pyruvate, apparently due to impaired carbohydrate oxidation following increased secretion of adrenaline (Beecher, Francis & Anfinsen, 1950; Brewster, Bunker & Beecher, 1952). At the comparatively low dosage levels of anaesthetic drugs now employed, such metabolic acidosis is not likely to be serious except in aggravating an existing acidosis, particularly in children or diabetic subjects, or possibly in patients who have had thoracic operations (Stead *et al.* 1953).

Metabolic alkalosis is not encountered as a consequence of anaesthesia. Although intravenous barbiturates are administered in a strongly alkaline solution, the dosage used in anaesthetic practice is too small to affect acid-base balance directly. Pre-existing metabolic alkalosis, as may arise from prolonged vomiting, may render a patient unduly sensitive to the effects of hyperventilation and delay the return of spontaneous breathing.

2. Changes in Cell Metabolism

a. Introduction

Direct studies of cell metabolism in man are difficult, but information can be obtained indirectly, for example from changes in the composition of body-fluids during anaesthesia. A change in concentration of a blood constituent, however, does not necessarily imply a change in its rate of production in the tissues, since haemodilution or concentration, interchange between fluid compartments, acid-base imbalance, changes in urinary or hepatic excretion, alterations in the rate of secretion into the alimentary tract, and many other factors, may produce marked variations in the composition of the plasma. The significance of an isolated finding such as, for example, an increase in the concentration of urea nitrogen in the blood during anaesthesia is difficult to assess without information about blood concentration and hepatic and renal function. For this reason in-vitro experiments on surviving animal tissues have up to the present been of greater value in determining the effects of anaesthetic drugs on cells than have clinical observations. On the other hand, many of these animal experiments have involved the use of anaesthetic drugs at toxic concentrations, and the changes observed may bear little relation to those occurring during surgical anaesthesia in man.

b. Oxygen Consumption

Measurements of changes in oxygen consumption during anaesthesia have yielded conflicting results. Brewster, Isaacs & Andersen (1953) recorded substantial increases in oxygen uptake in dogs during ether anaesthesia, whereas Shackman, Graber & Redwood (1951) found a mean decrease of 22% in 20 of the 23 patients during surgical operations under thiopentone, nitrous oxide, cyclopropane and curare in various combinations. Many of the patients in the latter series showed high oxygen uptakes before anaesthesia, probably due to apprehension, while, in the investigations of Brewster *et al.* (1953), adrenaline release during induction of anaesthesia could account for at least some of the increase over resting levels. On balance, it seems likely that, with adequate pre-medication and smooth induction and maintenance of anaesthesia with modern techniques, the oxygen requirements of an anaesthetized man are less than when he is awake and resting. Further, it has been shown by Shackman *et al.* (1951) that this decrease is not due to a reduction in the available oxygen in the blood, and a diminished utilization of oxygen by the cells must be assumed.

A moderate fall in cerebral oxygen consumption induced by narcotic drugs has been shown by many experiments. Quastel & Wheatley (1932) observed that the reduction in oxygen uptake by brain tissue exposed to various drugs roughly paralleled their anaesthetic potency. Himwich and co-workers (Himwich, Homburger, Maresca & Himwich, 1947) found that thiopentone caused a greater fall in oxygen consumption in cerebral cortical tissue than in lower brain centres, while steroids with narcotic properties inhibited glucose oxidation in brain tissue more than did other steroids (Gordon & Elliott, 1947).

The fall in oxygen consumption which occurs during the production of hypothermia is related to the fall in body temperature; whether the relationship is linear (Bigelow, Lindsay, Harrison, Gordon & Greenwood, 1950b) or exponential (Spurr, Hutt & Horvath, 1954) is not certain. No doubt because of the fact that the heart, though slowed, is still active at low body-temperatures, its oxygen requirements do not fall to the same extent as do those of other organs. Thus at 25° C., while total body and cerebral oxygen utilization are reduced to about one-third of the normal value, the cardiac oxygen consumption falls by only 50% (Neil, 1956). The ratio of oxygen used by the heart to total oxygen used by the body is therefore greater during hypothermia than at normal body-temperature.

Most observers agree that oxygen consumption in the brain, liver and splanchnic areas shows little change during controlled hypotension as compared with that during normotensive anaesthesia, provided that the fall in blood pressure is not extreme and a steep head-up tilt is avoided (for references see Little, 1956). Although several investigators found a decrease in the mean blood flow, this was compensated by vasodilatation, or by an increase in the arteriovenous oxygen difference, or both. The over-all fall in oxygen consumption observed by Lynn, Sancetta, Simeone & Scott (1952) during high spinal block was probably due to the accompanying general anaesthesia, for the one subject in whom no fall was observed received the smallest dose of thiopentone, and unanaesthetized patients in the control series also showed minimal reduction in oxygen uptake during high spinal analgesia.

c. Carbohydrate Metabolism

Since glucose is the principal source of energy for nervous tissue, attempts have been made to establish a common mode of action for anaesthetic agents as inhibitors of some stage in the provision of energy from oxidation of glucose. Dissociation of oxidizing from phosphorylating systems could interfere with the production of high-energy phosphorus compounds, such as adenosine triphosphate, and so with the transfer of chemical energy, thus accounting for a general depression of cell activity. Brody & Bain (1951, 1954) have shown that barbiturates can uncouple oxidation from phosphorylation, and Hulme & Krantz (1955) demonstrated a similar action by ether on brain tissue. Further, Hulme & Krantz (1956) have found that, for a number of barbiturates of widely differing anaesthetic potency, the ability to uncouple oxidation from phosphorylation closely parallels hypnotic efficiency. At concentrations in the anaesthetic range they observed from 10 to 15% uncoupling as compared with 20% for ether. A similar effect has been shown with anaesthetic steroids by Truitt, Bell & Krantz (1956). This subject has been reviewed by Brody (1955).

Anaesthetic drugs have other significant effects on carbohydrate metabolism. In 1912 Elliott showed that both ether and chloroform cause adrenaline release from the suprarenal medulla, leading to hydrolysis of glycogen in the liver and to hyperglycaemia (Cori, 1931). This release occurs only if the innervation of the suprarenals is intact (Brewster *et al.* 1952), while the incidence of early hyperglycaemia during ether anaesthesia is markedly reduced by the use of thiopentone for the induction of anaesthesia (Bass, Watts & Chase, 1953). More prolonged hyperglycaemia causing post-operative glycosuria may, however, occur due to glyconeogenesis from amino acids stimulated by an increased production of suprarenal cortical steroids (Long, Katzin & Fry, 1940; Hayes & Brandt, 1952). On the other hand, anaesthetic doses of thiopentone do not significantly affect levels of blood glucose in man (Sessoms, Watts, Chase & Andrews, 1955), although larger doses cause hyperglycaemia in dogs (Booker, 1946).

Hyperglycaemia as a sequel to the administration of curare to unanaesthetized animals has been described (McIntyre, 1947; Harroun, Beckert & Fisher, 1947); but it was probably due to excitement and sympathetic over-activity. Gray (1954) states that hyperglycaemia has not been observed following surgery in patients who have been given D-tubocurarine chloride, and considers that it may safely be employed in diabetic subjects. Laurence & Stacey (1952) and Griffiths (1953) have observed hypoglycaemia following the administration of hexamethonium. This they ascribed to blockade at the ganglia of sympathetic impulses to the liver and adrenal medulla. Whilst the sympathetic ganglion-blocking effects of muscle-relaxant drugs are weak in comparison with their action at the neuromuscular junction, they do have some effect on autonomic ganglia. This is most marked with D-tubocurarine. Thus, while in theory this drug might potentiate the hypoglycaemic effect of insulin, no practical problem in respect to this has yet appeared.

Raised levels of plasma lactate have been reported during anaesthesia in dogs both with thiopentone (Booker, French & Molano, 1949) and with ether (Bunker, Beecher, Briggs, Brewster & Barnes, 1951). The latter regard this as due to tissue anoxia. Plasma levels of pyruvate and of α-ketoglutarate fall rapidly in rats during barbiturate anaesthesia, the effect being reversed by adrenaline (Goodwin & Williams, 1952). Such changes may, of course, be due to factors other than the direct action of anaesthetic agents on cells, for example anoxia, hypercapnia, changes in cell pH, lack of co-factors for enzyme action, or intermediary metabolites. Thus Olsen & Fenn (1956) found that inhalation of carbon dioxide produced a rise in concentration of plasma lactate in the absence of anoxia. Hackel, Sancetta & Klenerman (1956) found no change in the coronary arteriovenous differences of glucose, lactate and pyruvate during hypotension induced by high spinal block in man, implying that, in the myocardial cell at least, hypotension does not significantly alter the pattern of carbohydrate metabolism.

d. Protein Metabolism

Changes in the metabolism of proteins and amino acids caused by anaesthesia alone are insignificant by comparison with the profound alterations in nitrogen metabolism induced by surgery. Alterations in concentrations of plasma protein are generally a consequence of altered fluid balance, while changes in the electrophoretic pattern of plasma proteins probably indicate some degree of damage to the liver rather than a general effect on protein metabolism.[1] Chloroform, as is well known, can seriously impair protein metabolism by a direct hepatotoxic action, but other anaesthetic agents are not noteworthy in this respect. Barbiturates cause a rise in plasma volume, apparently by a movement of fluid into the circulation (Bollman, Svirbely & Mann, 1938). Neither degradation of amino acids nor urea production is impaired by anaesthesia (Beecher, 1938).

3. Changes in Renal Function

In considering the action of anaesthetic drugs on the kidney, Orth & Cohen (1951) pointed out that effects commonly ascribed to toxic action, such as decreased urea clearance, decreased phenol red excretion, post-operative acidosis, raised level of non-protein nitrogen in the blood, and transient proteinuria, are more probably the result of extra-renal factors—vascular, respiratory or surgical—than of specific damage to renal cells. In their cases no significant difference was found in the incidence of such effects following chloroform and other anaesthetics. As these observations were made in the post-operative period, the effects of anaesthesia cannot be distinguished from those of surgery. There are, however, some studies in which this distinction can be made (Burnett, Bloomberg, Shortz, Compton & Beecher, 1949; Craig, Visscher & Houck, 1945; de Wardener, 1955). These show that with ether or cyclopropane there is a marked fall in urine flow, beginning almost immediately after the start of induction of anaesthesia and returning to nearly normal levels when consciousness returns. There is also a reduction in effective renal plasma flow during ether or cyclopropane anaesthesia, its extent depending on the plane of anaesthesia. Thus, in light anaesthesia with these agents, the renal plasma flow is reduced by about 30%, while in deep anaesthesia it is 60% below the normal value. The fall is not further reduced by prolongation of unconsciousness. The rate of glomerular filtration also falls. These changes follow arteriolar vasoconstriction in the kidney and are abolished by denervation. They are not affected by certain surgical procedures (Habif, Papper, Fitzpatrick, Lowrance, Smythe & Bradley, 1951), but injection of adrenaline or noradrenaline will increase vasoconstriction and thereby diminish the renal blood flow still

[1] See also Martin & Neuberger, *Brit. med. Bull.* 1957, 13, 113.—ED.

further. It is interesting to note that the pressor agent, methylamphetamine, did not diminish renal blood flow during anaesthesia (de Wardener, 1955).

No additional fall in renal blood flow was observed following the administration of pentamethonium to the anaesthetized subject (Miles, de Wardener, Churchill-Davidson & Wylie, 1952). This implies that compensatory renal vasodilatation occurs in response to the fall in blood pressure. However, it has been found that induced hypotension produces a transient fall in renal blood flow and a more lasting reduction in glomerular filtration rate, owing presumably to inadequate glomerular blood pressure. This was accompanied by retention of sodium and water and initially of potassium also (Moyer, Morris & Seibert, 1955; Moyer, McConn & Morris, 1955). In addition, Becker (1956) has shown a reversible decrease in phenol red excretion during controlled hypotension with trimetaphan in patients undergoing surgical operations under general anaesthesia. This indicates a diminished tubular excretory capacity, though not necessarily actual cell damage.

The changes just described in renal blood flow and in glomerular filtration rate during anaesthesia, alone and with hypotension, do not adequately explain the oliguria, since urine volume can be maintained in the face of greatly reduced glomerular filtration, by reduced tubular reabsorption of water. Active reabsorption of water is promoted by pituitary antidiuretic hormone (Smith, 1951) and it is likely that anaesthetic agents stimulate its production via the supra-optic hypophysial tract. This view is supported by the finding of an antidiuretic substance in human urine in the post-operative period (Eisen & Lewis, 1954). Bachman (1955) has, however, shown that the antidiuresis induced by ether or cyclopropane is not abolished by section of the pituitary stalk or of the hypothalamic-pituitary tract.

Page, Kupsinel & Adams (1954) have observed a decrease in glomerular filtration rate, tubular reabsorption, renal blood flow and urine volume during hypothermia. The same changes have been found by Nowill, Hall, Ausherman, Smith, Shingleton & Boyarsky (1956) during induced hypothermia in dogs, but these investigators did not find any consistent pattern of change in extraction ratios.

Changes in renal function during general anaesthesia with and without controlled hypotension appear to be primarily due to haemodynamic factors rather than to a direct effect of the anaesthetic agent on kidney cells. During hypothermia, however, the general reduction in cell activity may also play a part, particularly on tubular secretion and reabsorption, since glomerular filtration is passive.

4. Changes in Hepatic Function

Chloroform is a notorious hepatotoxic agent, the characteristic lesion in acute chloroform poisoning being centrilobular necrosis. The severity of the damage is related to the concentration and duration of exposure to the drug and also to the state of oxygenation and the nutrition of the patient (Himsworth, 1950). Thus substantial protection is afforded by adequate oxygenation (Goldschmidt, Ravdin & Lucké, 1937) and by good nutritional status in respect of protein, carbohydrate and lipotropic factors (Goldschmidt, Vars & Ravdin, 1939; Miller & Whipple, 1940, 1942). Other anaesthetic agents, notably divinyl ether, produce effects on the liver somewhat similar to those of chloroform (Goldschmidt, Ravdin, Lucké, Muller, Johnston & Ruigh, 1934), although diethyl ether, nitrous oxide and cyclopropane rarely cause more than transient disturbances (Rosenthal & Bourne, 1928; Raginsky & Bourne, 1934).

In a recent re-evaluation of chloroform as an anaesthetic agent, Orth, Pohle & Sims (1951) have compared the effects of this drug on the liver with those of several other agents, including ether, cyclopropane, bromethol and thiopentone, in 121 patients following major surgical operations. Using a battery of five standard liver-function tests, these workers obtained evidence of hepatic dysfunction in 52% of the cases anaesthetized with chloroform and in 44% of the control series. Further, frank jaundice occurred in 6 of the 65 patients in the group receiving chloroform as compared with 3 of the 56 who had received other anaesthetic agents. Waters (1951) states that, if chloroform is to be retained as an anaesthetic for surgical operations, accurate adjustment of the vapour concentration and also adequate oxygenation and carbon-dioxide elimination are essential. This latter point is reinforced by the report of Siebecker & Orth (1956) on serious hepatic damage in patients subjected to thoracotomy under chloroform anaesthesia.

The recently introduced fluorinated volatile anaesthetics, trifluoroethyl vinyl ether (Fluoromar) and the fluorinated hydrocarbon $CF_3CHClBr$ (halothane) do not so far appear to resemble chloroform in hepatotoxic properties. Gainza, Heaton, Wilcox & Virtue (1956), in comparing Fluoromar with other anaesthetic agents, found similar evidence of minor hepatic dysfunction. Raventós (1956) found no significant changes in hepatic function in rats and dogs anaesthetized with halothane, although "minor" histological changes in the liver were noted. In a series of patients anaesthetized with halothane on whom several liver function tests were carried out, we have observed no greater incidence of post-operative impairment of liver function than in the patients of a similar series who were given other anaesthetic drugs.

The difficulty of assessing the part played by the anaesthetic agent in producing biochemical disturbances following surgical operations is nowhere more real than where it concerns the interpretation of liver function tests. Nevertheless, the numerous reports of abnormal results leave little reason for doubt that impairment of liver cell activity follows the use of many anaesthetic agents besides chloroform, although this is frequently transient and unaccompanied by clinical evidence of hepatic disturbance. The effects observed include reduction in the liver's capacity to excrete bilirubin, bromsulphthalein and other dyes, to convert glycogen and amino acids to glucose, to oxidize fats, to maintain normal plasma protein ratios (both as determined by electrophoresis and by turbidity and flocculation reactions), to convert lactate to glycogen, to metabolize drugs and to synthesize enzymes and prothrombin.

These diverse effects of anaesthetics on hepatic function point to an action on some pathway involved in nearly all cellular activities, such as an energy-yielding mechanism. In support of this view, Shackman, Graber & Melrose (1953) found that anaesthesia produces a fall in oxygen consumption by the liver and splanchnic viscera that is proportionately greater than the fall in oxygen uptake of the body as a whole. Further, Christie & Judah (1954) and Dianzani (1955) have produced evidence of structural changes in liver mitochondria[1] and of dissociation of oxidation from phosphorylation which

[1] See also Stoner & Magee, *Brit. med. Bull.* 1957, 13, 102.—Ed.

could explain the numerous effects on liver cell activity produced by anaesthetic drugs.

The effects on liver function of hypotension induced by high spinal analgesia in anaesthetized subjects have been studied by Lynn *et al.* (1952) and by Greene, Bunker, Kerr, von Felsinger, Keller & Beecher (1954). The former group found retention of bromsulphthalein up to seven days following operation, whereas the latter were unable to demonstrate any correlation between bromsulphthalein excretion by the liver and the degree of hypotension. Greene and his colleagues further observed that, even in patients with pre-existing liver disease, post-anaesthetic levels of serum bilirubin and alkaline phosphatase were similar to those following ether or cyclopropane anaesthesia.

Hypothermia has been found to cause retention of bromsulphthalein (Brokaw & Penrod, 1949; Nowill *et al.* 1956), possibly due to a fall in hepatic blood flow, but the impairment was fully reversible. Hyperglycaemia followed by hypoglycaemia was reported by Fuhrman & Crismon (1947) and attributed to exhaustion of the liver glycogen stores, while the fall in the blood-urea level during hypothermia described by Smith & Fay (1940) and by Nowill *et al.* (1956) is presumably due to a reduced rate of amino-acid catabolism in the liver at low body-temperatures. Thus, although anaesthetic agents in general, and special techniques employed in modern anaesthetic practice, do produce some degree of functional impairment in the liver, this is usually only temporary, is fully reversible, and is not associated with clinical symptoms. The risk of aggravation of pre-existing liver disease by anaesthesia can be minimized by careful preoperative attention to the patient's nutritional state, by keeping the dosage of the anaesthetic drugs as low as possible and by ensuring adequate pulmonary ventilation throughout the operation.

5. Changes in Water and Electrolyte Balance[a]

Unless fluids are administered parenterally to an anaesthetized patient, his water and salt balance is inevitably negative. Quite apart from any operative blood loss, the magnitude of the loss of fluid and of electrolytes varies with perspiration, pulmonary water loss, and urine volume. Urine output during anaesthesia is usually small, for reasons discussed in the section on renal function. In the immediate postoperative period marked retention of water, sodium and chloride occurs with an increase in body-weight, but the urinary loss of potassium rises. These changes are characteristic of a general response to trauma rather than to anaesthesia (Wilkinson *et al.* 1949, 1950b; Moore & Ball, 1952; Le Quesne & Lewis, 1953). The additional fluid is probably retained in the extracellular space (Lyon, Freis & Smithwick, 1949). Le Quesne (1957) considers that water and sodium retention should be distinguished, the former being due, initially at least, to production of antidiuretic hormone under anaesthetic stimulation of the pituitary, and the latter to increased production of adrenocortical steroids following surgical stress. (See also Dudley, Boling, Le Quesne & Moore, 1954.)

a. Fluid Shifts

Anaesthesia per se produces recognizable alterations in body-fluids. It has been known for many years that ether or cyclopropane causes a fall in circulating blood volume with increased haematocrit values (Barbour & Bourne, 1924; McAllister, 1938; Stewart & Rourke, 1938; Fay, Andersch & Kenyon, 1939). These changes are usually attributed to an increased number of circulating erythrocytes, following contraction of the spleen. Stewart and Rourke, however, found an increase in extracellular fluid (ECF) volume following ether anaesthesia; movement of fluid out of the circulation and into the ECF may therefore be responsible for the changes in haematocrit values. Barbiturates, on the other hand, cause haemodilution with a reduction in ECF volume (Bourne, Bruger & Dreyer, 1930). Since body-fluid changes are invariably accompanied by movement of electrolytes, these water shifts may well be secondary to movements of sodium or potassium. Further, they may occur without marked change in the concentrations of electrolytes in the plasma.

Extensive fluid shifts have also been recorded in hypothermia. Hegnauer, Shriber & Haterius (1950) found a rise in haematocrit values and blood viscosity in dogs cooled to 20° C., while Rodbard, Saiki, Malin & Young (1951), also in animal experiments, found a reduction of just over 30% in plasma volume. In their experiments there was a reduction in the ECF volume as measured by thiocyanate, but no change when measured by inulin. They conclude that the blood is pooled in some vascular reservoir, while Nowill *et al.* (1956) postulate accumulation in the lungs to account for the observed changes.

b. Electrolyte Changes

Changes in plasma electrolyte concentrations during uncomplicated anaesthesia are small, but may become important if either acidosis or alkalosis develops. Thus, Young, Sealy & Harris (1954) and Brown (1955) found a marked rise in plasma potassium when carbon-dioxide retention was permitted, while in respiratory alkalosis a slight fall in potassium concentration is common. Administration of adrenaline to the anaesthetized dog also produced a marked elevation of plasma potassium (O'Brien, Murphy & Meek, 1953). The possible role of hyperkalaemia in the production of ventricular arrhythmias during anaesthesia is discussed by these authors.

The hypothermic state is associated with disturbance of electrolyte concentrations, a rise in plasma potassium having been recorded by Elliott & Crismon (1947) and by Bigelow and his group (Bigelow, Lindsay & Greenwood, 1950a) and a fall by Swan *et al.* (1953) and Nowill *et al.* (1956). Boeré (1956a) also recorded a fall during hypothermia, but found a significant association between carbon-dioxide retention and hyperpotassaemia. The association of ventricular fibrillation with carbon-dioxide retention during hypothermia may therefore be due to the known effect of high levels of plasma potassium in causing increased neuromuscular excitability. The additional potassium is presumably derived from tissue cells, and such cellular loss of potassium may conceivably be due to an effect of hypothermia on the oxidation-reduction mechanism which normally maintains the electrolyte concentration gradients between cells and ECF (Carey & Conway, 1956).

Changes in plasma sodium and chloride during hypothermia are slight (Woodruff, 1941; Swan *et al.* 1953; Nowill *et al.* 1956). Elliott & Crismon (1947) observed a small but statistically significant rise in serum calcium, whereas Bigelow *et al.* (1950a) found no consistent change.

In induced hypotension Vitale (1956) has found in 70% of

[a] See also Black & Emery (*Brit. med. Bull.* 1957, 13, 7) and Wrong (*Brit. med. Bull.* 1957, 13, 10).—Ed.

cases a rise in plasma potassium after administration of an unspecified curare drug (probably D-tubocurarine). Since potassium salts are known to antagonize curare (Wilson & Wright, 1936) and since normal neuromuscular excitability is greatly dependent on normal potassium concentrations in plasma and ECF, it is specially relevant that potassium-depleted patients show an increased sensitivity to muscle relaxants (Foldes, Hunt & Ceravolo, 1952; Foster, 1956).

6. Relation of Endocrine Factors to Metabolic Changes associated with Anaesthesia

The production of adrenocortical hormones is increased in response to many forms of "stress". Agents which evoke this non-specific reaction are those which disturb homeostasis and therefore include anaesthesia and surgical trauma. The primary biological purpose of the endocrine disturbance induced by these stimuli is to raise the body's resistance, and for this adrenocortical hormones appear to be necessary.

Many of the metabolic changes which have been observed following anaesthesia and surgery, in particular the negative nitrogen balance, sodium and water retention and increased potassium excretion, bear a striking resemblance to those produced by hyperactivity of the adrenal cortex. Indirect evidence of a possible relationship between the stresses of anaesthesia and surgery and a subsequent increase in the production of adrenal hormones is offered by the eosinopenia which follows operation (Hayes, 1952). Many observers have reported an increase in plasma and urine concentrations of corticosteroids in the post-operative period (Stewart, 1955), but only a few have made these direct measurements following anaesthesia. Such observations as are available are somewhat conflicting and suggest that the type of anaesthetic drug used may be an important factor. Thus, Sandberg, Eik-nes, Samuels & Tyler (1954) found an increase in the plasma concentration of free 17-hydroxycorticosteroids following the induction of general anaesthesia with thiopentone, ether and cyclopropane in eight patients, while Beigelman, Slusher, Slater & Roberts (1956) observed that ether, but not pentobarbitone sodium, resulted in a marked early rise of corticosteroids in the adrenal venous effluent of the rat. The latter group believed that the similarity of corticosteroid values obtained with both types of anaesthetic drugs in later collection periods was the result of the continued stresses of cannulation and blood loss. Virtue & Helmrich (1956) found no significant difference in free plasma dihydroxycorticosteroid levels measured a few days before operation, immediately before induction of anaesthesia, and in 36 out of 47 cases following one hour of anaesthesia with cyclopropane (10 cases), nitrous oxide–thiopentone (10 cases) and ether (16 cases). In the other 11 cases in the series, all of whom were receiving ether, the blood steroid values obtained were significantly higher than those in the pre-anaesthetic periods. These values continued to rise during surgery in proportion to the degree of trauma and reached a maximum some time after the end of the operation. Moore's group (quoted by Virtue and Helmrich) also found that blood steroid values were unchanged during nitrous oxide–thiopentone anaesthesia but, unlike Virtue and Helmrich, they reported increases when cyclopropane was the agent used. It would seem, from the scanty data available, that some anaesthetics may cause an increase in the blood corticosteroid values but that these are likely to be much lower than the values found after surgical trauma. If, as has been suggested, the blood corticosteroid changes can be related to the metabolic disturbances observed after surgical operations, it would appear that the part played by the anaesthetic agent is small compared with that due to surgery.

It is likely that most forms of stress, including anaesthesia, cause a general increase in sympathetic activity with release of adrenaline into the blood and increased production of adrenocorticotrophic hormone (ACTH) by the anterior pituitary gland. This in turn stimulates secretion of adrenocortical hormones by the adrenal glands. It has been suggested, however, that there is another factor which may play a part in controlling ACTH release—the blood level of adrenocortical hormone before the stress. Thus, if this is already high, as in patients who have been under treatment with cortisone before coming to operation, adrenal cortical function may be depressed and may fail to respond normally to stress stimuli. Such patients may, during anaesthesia and surgery, develop a shock-like state which fails to respond to conventional forms of treatment, but which improves immediately when hydrocortisone is given by intravenous infusion (Lundy, 1953).

Further evidence of increased endocrine activity associated with anaesthesia and surgery is given by Llaurado (1955) who reported an increased urinary excretion of aldosterone, and by Eisen & Lewis (1954) who, as already mentioned, found in the urine of patients following operation an antidiuretic substance which was probably posterior pituitary antidiuretic hormone. Thus it would appear that endocrine factors are of considerable importance in the production of the biochemical disturbances which follow anaesthesia and surgery. However, it should be noted that, even where adrenocortical activity has been suppressed, the usual post-operative metabolic response can still occur (Robson, Horn, Dudley & Stewart, 1955).

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THE MYONEURAL BLOCKING ACTION OF ANAESTHETIC DRUGS

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1 Diethyl ether and drugs used in anaesthesia
2 Other volatile anaesthetics
3 Gaseous anaesthetics
4 Intravenous anaesthetics
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6 Clinical applications
References

Now that anaesthetists handle the problems of relief of pain, of sleep, and of relaxation of skeletal muscle as separate parts of the process of clinical anaesthesia, it is necessary that any interactions between the drugs used for one or other aspect of the whole be appreciated. This brief review concerns the third of these aspects of the process of anaesthesia.

1. Diethyl Ether and Drugs used in Anaesthesia

Ether has a peripheral curariform effect which seems to have been described first by van Leeuwen (1913). Simonart & Simonart (1935) showed that ether reduced the contraction of skeletal muscle produced by injection of acetylcholine, and Brown, Dale & Feldberg (1936) confirmed this. Poulsen & Secher (1949a) demonstrated that the threshold values for effective indirect stimulation of a motor nerve were raised some three times by therapeutic amounts of ether. According to Secher (1951c), who carried out a number of studies with isolated nerve-muscle preparations (Secher, 1950, 1951a), the peripheral action of ether is primarily curariform and to a much lesser degree a depression of reactivity in the muscle fibre or of conduction in the motor nerve, a point made by Forbes, McIntosh & Sefton in 1916. Naess (1950a, 1950b) described a biphasic effect of ether on the excitability of nerve: increase during induction and decrease later. Much of the effect of ether on muscle tone is due to depression of central nervous system activity, particularly reflex activity in the spinal cord (Austin & Pask, 1952). Graham & Heathcote (1952) showed that ether in relatively large amounts has no effect on the extensibility or elastic recoil of skeletal muscle groups.

The increasing use of intravenous procaine during a variety of surgical procedures when diethyl ether and/or relaxant drugs may be employed (Mushin & Rendell-Baker, 1949) serves as a reminder that the local anaesthetics can increase the paralysing effect of curariform drugs by reducing the output of acetylcholine at motor nerve-endings. This may be of significance when patients suffering from subclinical myasthenia gravis are subjected to these agents, or if cocaine derivatives are used by infiltration in myasthenic patients under the influence of neostigmine (Jaco & Wood, 1944). Nicholls & Quilliam (1956), as a result of electrical studies on frog muscle, came to the conclusion that procaine also acts as a competitive or curariform inhibitor.

The ganglion-blocking agents (see Paton & Zaimis, 1952) do not have any marked neuromuscular activity, though Randall, Peterson & Lehmann (1949) described a slight blocking effect of thiophanium (Arfonad) in dogs. There is no mention of activity being affected by anaesthetics, but incompatibility of solutions of thiophanium with thiopentone or gallamine is marked (Scurr & Wyman, 1954).

Of the substances used for sedation and analgesia, synergism exists between the opium alkaloids (e.g. morphine and codeine), or the synthetic analgesics pethidine and methadone, and curariform drugs, in mice (Lang, Kimura & Unna, 1951) but not necessarily in other animals (Pick & Richards, 1947). It is assumed that this action is central, but Kopera & Armitage (1954) have reported that chlorpromazine, pethidine and promethazine inhibit the response of cat gastrocnemius to direct or indirect stimulation. Despite the wide-spread use of pethidine as a supplement during anaesthesia, no report of such action in man is available. An additive effect of ether and pethidine or thiopentone and pethidine on muscle is to be expected. Nalorphine does not appear to antagonize this action of pethidine. Chlorpromazine increases the action of gallamine and curare in paralysing small animals and also increases the hypnotic effect of narcotics and ether (Courvoisier, Fournel, Ducrot, Kolsky & Koetschet, 1952; Burn, 1954); but whether it increases the curariform action of the anaesthetic does not seem to have been reported, though it is

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probable. The noticeable immobility of patients who have been given chlorpromazine may be in part due to its curariform action. Quilliam (1955a) and Nicholls & Quilliam (1956) describe a neuromuscular block in frog muscle by paraldehyde and methylpentynol, which is attributed to reduction of acetylcholine released at the nerve ends. Chloral hydrate does not have a relaxant action, but bromethol does (Gross & Cullen, 1943).

Anticholinesterases are used to cut short the effect of curarizing agents such as D-tubocurarine and gallamine. Poulsen & Secher (1949b) showed that neostigmine antagonizes the curariform activity of ether, and Secher (1951b) confirmed this, though the demonstration was confused by the persistent depolarizing effect of excessive amounts of the anticholinesterase.

2. Other Volatile Anaesthetics

Secher (1950, 1951d), who reviewed the scanty early literature, attributed to chloroform an action similar to that of ether but weaker. Low concentrations of chloroform had a stimulant effect on the contraction of the frog rectus muscle caused by added acetylcholine (Torda, 1943), and high concentrations diminished the response or caused contracture. Physostigmine increased the response to acetylcholine of muscle treated with chloroform or ether. Chloroform does not increase the effect of curariform drugs (Lang *et al.* 1951), nor does it affect the depolarizing relaxants or the action of mephenesin. Some attempt to elucidate the complex mode of action of chloroform has been made by Miguel (1946) who confirmed earlier reports that chloroform inhibits cell cholinesterase (as does ether) but not plasma cholinesterase, except in abnormally high concentrations.

Divinyl ether produces poor clinical relaxation. No detailed studies of its action have been found, other than the report by Lang *et al.* (1951) of its synergism with D-tubocurarine in mice. It is not an anticholinesterase (Adriani, 1941). Trichloroethylene does not produce good muscular relaxation and again there appears to be a dearth of experimental work. A similar situation is found with ethyl chloride.

Fluothane[1], a non-explosive fluorine containing volatile hydrocarbon anaesthetic, has been the subject of recent investigation (Raventós, 1956; Burn, Epstein, Feigan & Paton, 1957). It exerts by itself very little ganglion-blocking action but increases the action of hexamethonium and of D-tubocurarine. The neuromuscular blocking action of Fluothane is minimal, but that of competitive relaxants is greatly increased and that of suxamethonium antagonized. As a result the dosage of D-tubocurarine needed to produce adequate relaxation should be reduced. Vagal activity is retained or even enhanced during Fluothane anaesthesia, and bradycardia may be marked; full doses of atropine should therefore be given, especially if neostigmine is to be used to restore neuromuscular transmission. Gallamine, which has no appreciable ganglion-blocking activity and which inhibits vagal activity, is the best relaxant to use with this anaesthetic.

3. Gaseous Anaesthetics

There are few reports of studies with gaseous anaesthetics on the muscular system (Naess, 1950c). Nitrous oxide produces jactitation when given in excessive concentrations, but this phenomenon appears to be due entirely to cerebral anoxia (Clement, 1951). Relaxation of muscles under " gas " is poor.

Ethylene produces better relaxation but no peripheral action has been demonstrated for it or for cyclopropane (Gross & Cullen, 1943). Neither of these gases is an anticholinesterase (Adriani, 1941), but ethylene increases the relaxant effect of decamethonium (Harris & Dripps, 1950). Possible synergism with suxamethonium does not seem to have been reported. Cyclopropane does not affect competitive or persistent depolarizing relaxants in mice (Lang *et al.* 1951).

4. Intravenous Anaesthetics

The effect of intravenous barbiturates on muscular activity has been investigated with some thoroughness, and the literature on the subject is reviewed by Sirnes (1954). In anaesthetic concentrations, barbiturate has no effect on conduction in the motor nerve (Comroe, Dripps, Botello & Metz, 1947), but exerts a mixed effect on muscular activity. Initially there is a degree of stimulation which is more apparent in isolated preparations than in intact muscles, affecting twitch responses more than sustained tetani, and which is present whether the muscle is stimulated directly or through its motor nerve. This action may cause a transient antagonism to relaxant drugs or to the depression caused by excess of magnesium ions. Very soon the main effect is seen, i.e., a depression of muscular activity which causes a synergism with all relaxants. Quilliam (1955a, 1955b) has done much to clarify the apparent contradiction by his electrical studies. The initial increase in twitch responses in rats (stimulant action) is accompanied by a decrease in voltage and a slowing of propagation of the action potential. It is thus a paradoxical result of depression of the muscle fibre. The depression has been demonstrated in various preparations, using unnaturally large doses of barbiturate, by Secher (1951a, 1951b, 1951d) among others.

As the depression affects the neuromuscular junction as well as the muscle fibre, the potency of curariform drugs and of persistent depolarizers is enhanced. Arrowood (1951), however, states that thiopentone diminishes the effectiveness of benzoquinonium (Mytolon) which is a relaxant which exerts competitive and mixed effects. It is obvious, therefore, that barbiturates depress muscular function in a complex manner. The interaction of relaxant drugs and barbiturates seems to apply only to the peripheral action, despite various theories to the contrary. Solutions of suxamethonium and thiopentone are incompatible.

5. Nutritional Factors

Any circumstance which debilitates muscle or weakens neuromuscular conduction will enhance the blocking effect of anaesthetics. Thus severe states of potassium deficiency, calcium lack, low blood volume, anoxia, or retention of carbon dioxide may be expected to increase their effect. In conjunction with synergistic relaxants or other combinations the results may be serious. Scurr (1954) has reported a prolongation of the effect of decamethonium in a child who was suffering from retention of carbon dioxide, and the problem of neostigmine-resistant curarization has recently attracted attention (*British Medical Journal*, 1957). The amount of neostigmine needed to cause paralysis by persistent depolarization

[1] Halothane has recently been adopted by the British Pharmacopoeia Commission as the approved name for this substance.—ED.

is much greater normally than that required to antagonize an effective dose of gallamine, but patients may vary widely in this respect.

6. Clinical Applications

The most obvious clinical application of the information reviewed is that volatile or injected anaesthetics produce muscular relaxation, not only from depression of central activity but also from peripheral actions. The most active is ether. The gases are a notable exception. Usually it is the curariform type of relaxant which is reinforced (D-tubocurarine and ether, Cole, 1945; gallamine and ether, Mushin, Wien, Mason & Langston, 1949) and there are few reports of interference with persistent depolarizing agents. Myasthenia gravis is an obvious danger with ether or thiopentone (Dundee 1952). Sedation with "tranquillizers" or barbiturate, analgesia with pethidine, and the use of suxamethonium followed by gallamine, especially if there is any subsequent giving of anticholinesterase, adds to the complexity of the pattern, so that the relatively weak depressant action of anaesthetics on the neuromuscular conduction or on the muscle fibre may suddenly become of importance. The possibility of potentiation of ganglion-blocking activity in D-tubocurarine with Fluothane (Johnstone, 1956) illustrates the need for constant awareness of drug relationships by the anaesthetist.

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Canadian Journal of Surgery
Journal Canadien de Chirurgie

The first number of this new quarterly journal of surgery appeared in October 1957. It is intended that the journal shall carry original articles, case reports, editorials, and book reviews in the two official languages of Canada—French and English; original articles in English will carry a summary in French, and vice versa. The historical section will feature the history of Canadian surgery.

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PRINCIPLES OF INHALERS FOR VOLATILE ANAESTHETICS

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b Vapour pressure and molecular weight
c Area of evaporation
3 Principles and performance of Snow's ether inhaler
4 Levy's quantitative chloroform apparatus
5 Ratio of flow-rate to concentration characteristic of the vaporizing chamber
6 Inhalers with the anaesthetic liquid kept at constant temperature
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8 Automatic thermo-compensation in anaesthetic inhalers
9 Other aspects of inhaler design
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The demand for applying standards and measurements common in engineering and instrument practice to anaesthetic apparatus also has gathered momentum only during the past few years. Quite recently an authoritative appeal (*British Journal of Anaesthesia*, 1957) has stressed the need for a fresh outlook on standards and measurements of anaesthetic appliances. A justified complaint frequently made is that technical details or data on performance of many a new anaesthetic apparatus are often unobtainable.

One purpose of these notes is to describe some features of anaesthetic inhalers and vaporizing bottles for which scientific and technical information has become increasingly available during the last two decades.

1. Inhalers amongst Quantitative Anaesthetic Appliances

In many respects, draw-over inhalers have led a Cinderella existence compared with other anaesthetic apparatus. For many decades the efforts of the anaesthetic profession and medical instrument suppliers have been directed to making intravenous anaesthesia a quantitative procedure by means of accurately calibrated syringes. Again, since the introduction of mixtures of oxygen and nitrous oxide and of other gases as everyday tools of the anaesthetist, continued improvements of flowmeters have been carried out—even if the development proceeded far from smoothly since Neu first used Küpper's flowmeter on an anaesthetic apparatus in 1910 (Neu, 1910). Thus it would have been unthinkable for anyone to inject intravenously a solution of unknown strength with a non-graduated syringe, or to administer oxygen–cyclopropane mixtures without any flowmeters, yet vaporizing bottles and draw-over inhalers for volatile anaesthetics were used for many years with practically no knowledge of the mixtures delivered by them. Granted a very few, notable exceptions, this was the general attitude prevailing at least during the first three or four decades of this century, and although a different outlook has developed during the past twenty years, there is even now a wide-spread tendency to deny such vaporizers the same rights as were granted long ago to metering devices for gas mixtures or to syringes. An argument that draw-over inhalers for ether anaesthesia and Trilene analgesia, or breath-triggered apparatus for nitrous oxide, are quite outmoded, can scarcely be sustained when recalling the work at many anaesthetic centres during the past decade, or even the numerous recent investigations on Fluothane on both sides of the Atlantic.

2. Factors Influencing Evaporation of Liquid Anaesthetics

Draw-over inhalers for the administration of anaesthetic vapour–air mixtures by the inspiratory effort of the patient have an honourable tradition beginning with the various ether

FIGS. 1, 2, 3. SCHEME ILLUSTRATING THE EFFECTS OF HEIGHT, AND OF SPEED OF GAS FLOW ABOVE THE VOLATILE LIQUID, ON THE VAPOUR CONCENTRATION IN THE MIXTURE LEAVING THE VAPORIZING COMPARTMENT

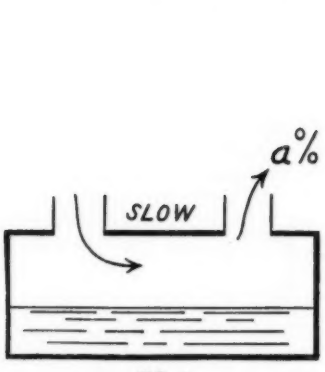


FIG. 1

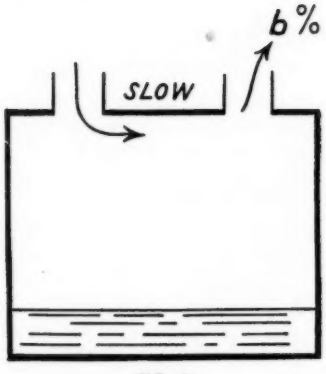


FIG. 2

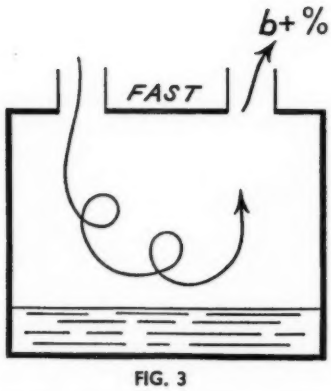


FIG. 3

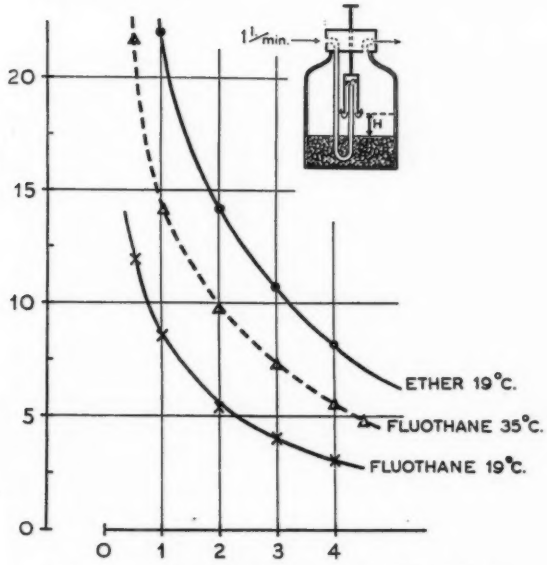
a%, b%, b+%: vapour concentration by volume. For full explanation of scheme, see text, p. 19.

inhalers designed by Snow[1] in 1847 (Snow, 1847a). Some principles of the design of efficient inhalers were already realized then, although possibly based more on intuition than on experimental evidence. Even nowadays a short discussion of the evaporation processes in a vaporizer may not be superfluous. If air is passed with a constant (low) velocity at some distance above the level of the liquid anaesthetic (fig. 1), it will pick up a certain amount of vapour so that the mixture leaving the vessel has a vapour strength of $a\%$ by volume. The concentration depends on the vapour pressure of the liquid at that temperature, the surface area, the velocity of the gas stream and on other factors. If the same flow is passed over the same liquid contained in a deeper vessel (fig. 2) so that the height of the flow above the level is raised, the concentration in the outflow ($b\%$) will be smaller than with the shallower vessel ($b < a$). If the flow-rate is now increased (fig. 3), the concentration ($b+\%$) may be considerably increased. However, in a simple vaporizing bottle, the high concentration will be maintained for only a short period owing to the rapid fall in temperature and the associated drop in vapour pressure of the liquid.

a. Pattern of Gas Flow through the Vaporizing Chamber

These general statements may be illustrated by the following example. A flow of 1 l./min. oxygen is passed through a "Boyle-type" vaporizing bottle so that it passes at a height

FIG. 4. VAPOUR CONCENTRATIONS IN GAS FLOWING AT VARIOUS HEIGHTS ABOVE THE LIQUID ANAESTHETIC

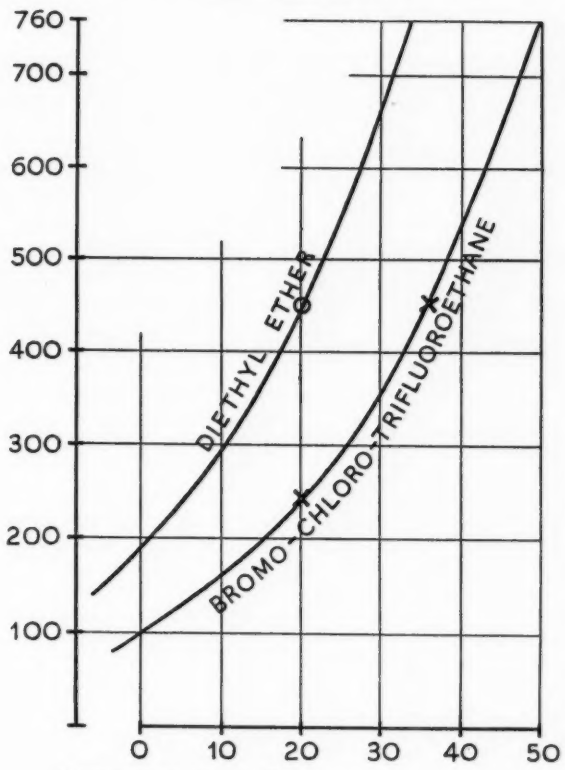


Abscissae: height (H) of gas stream above liquid (cm.)
Ordinates: vapour output (vol. %)

A Boyle-type vaporizing bottle illustrates the experimental arrangement, but special measures have to be taken to maintain constant temperature.

[1] See also "The evolution of anaesthetic apparatus: a brief pictorial survey", by A. Charles King, *Brit. med. Bull.* 1946, 4, 132.—ED.

FIG. 5. VAPOUR PRESSURE CURVES FOR ETHER AND FLUOTHANE



Abscissae: temperature (°C.)
Ordinates: pressure (mm. Hg)
Upper curve: diethyl ether
Lower curve: Fluothane (bromo-chloro-trifluoroethane)

The points referring to the experiments discussed in the text are marked by o and x on the curves.

of 3 cm. above the liquid ether; the outflowing mixture contains approximately ($a =$) 10% v/v of ether vapour. Next, the height of the flow is increased to 4 cm. above the level, and the concentration in the outflow is reduced thereby to ($b =$) 8%. (See uppermost curve in fig. 4.) If the flow-rate is raised to 4 l./min., the output can exceed ($b+ =$) 20% for a short time; then the temperature begins to fall rapidly and within 15 minutes the concentration may be down to one half of its initial value.

b. Vapour Pressure and Molecular Weight

If two different anaesthetic liquids are vaporized under otherwise identical conditions, it can be shown that the vapour pressures are not the only factors determining the vapour strength of the mixtures leaving the bottles. When a slow flow of oxygen is passed over ether at 20° C., the output may contain 10.3% v/v ether vapour. When the same experiment is repeated with Fluothane[2] under identical conditions, the output will contain 3.6%, so that the concentrations are in the

[2] Halothane has recently been adopted by the British Pharmacopoeia Commission as the approved name for this substance.—ED.

FIGS. 6, 7. SCHEME ILLUSTRATING THE INCREASE IN CONCENTRATION OF VAPOUR CARRIED AWAY BY A GAS STREAM WHEN THE EVAPORATING AREA IS ENLARGED

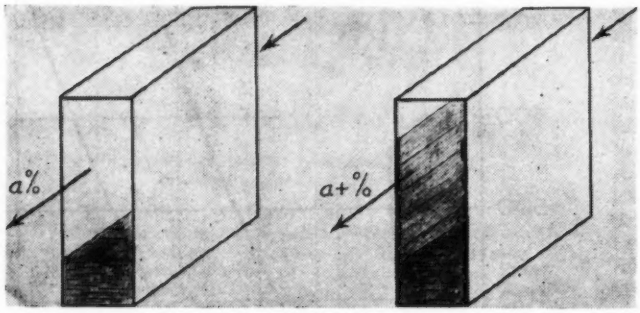


FIG. 6 FIG. 7

The surface of free liquid remains unaltered. For full explanation, see text.

ratio of 2.8:1. The vapour pressures of the two liquids (fig. 5) are 460 mm. and 240 mm. Hg, i.e. as 1.9:1. Much less Fluothane evaporates than could be expected from its lower vapour pressure. A more direct comparison is possible by repeating the experiment with Fluothane at 35° C. where its vapour pressure is equal to that of ether at 20° C.; the resulting concentration in the output rises to 6.5% v/v. The ratio of ether vapour to Fluothane vapour in the output when both liquids have the same vapour pressure is as 10.3: 6.5, or 1.6:1. For the low flow-rate (1 l./min.) and the height of the flow (3 cm.) above the liquid in these experiments, a simple theory (Chaplin & Cartwright, 1937) holds approximately, according to which the vapour strength of the mixture leaving the bottle is inversely proportional to the square root of the molecular weight. The molecular weights of Fluothane and ether are 198 and 74, or as 2.7:1; the square root of this ratio is 1.6 and this is about the ratio of the concentrations mentioned earlier on. Such simple results can be obtained only if the temperature of the liquids does not change during the experiment.

c. Area of Evaporation

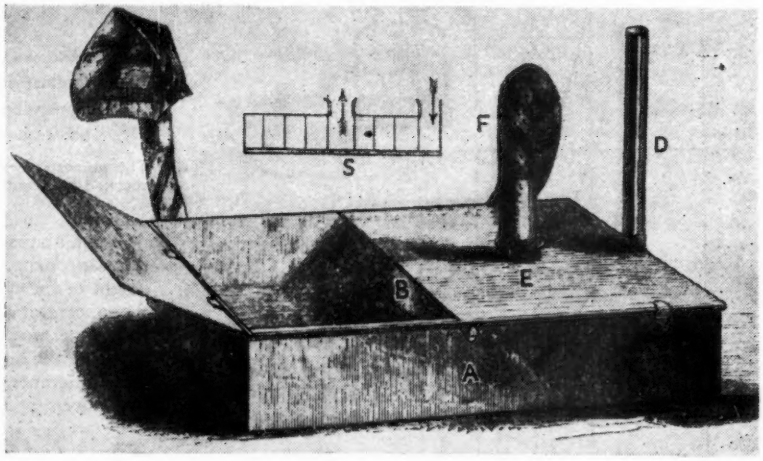
The size of the area from which the anaesthetic can evaporate also influences the vapour strength in the mixture leaving the apparatus. One old-established method of increasing the area utilizes various kinds of wicks (capillary-active material) dipping into the anaesthetic and sucking up some of the liquid to a certain height above the level. In the schematic arrangement of fig. 6 the gas stream passing through the rectangular box at a certain height above the liquid level picks up a certain concentration of vapour (a%). After covering the walls with wicks (fig. 7), and leaving all other conditions unchanged, the concentration in the outflow is increased to $a+$%. Such artificial evaporation surfaces are not evenly covered with liquid; they are "starved" at increasing heights and this is indicated by the graded shading of the walls. As early as 1848, Snow's chloroform inhaler (Snow, 1848) utilized a cylinder of blotting paper to increase the surface, while numerous designs of vaporizing bottles still in use incorporate a cylindrical wick, usually suspended in the middle of the bottle.

3. Principles and Performance of Snow's Ether Inhaler

The uptake of vapour into the gas stream can also be increased by prolonging the time during which a given volume is in contact with the liquid. This method was used in the various ether inhalers designed by Snow, where the path of air is lengthened considerably by forcing it to travel in a spiral track over the liquid ether. In the last of his ether inhalers (Snow, 1847b) air enters through the tube (D) ($\frac{5}{8}$-inch bore[3]) into the outermost spiral path in the vapour chamber as shown by the arrow in section S (fig. 8); it then travels gradually along the spiral towards the centre and out through the lid (E) into the breathing tube (F) ($\frac{1}{4}$-inch bore). The round vapour chamber (B) is 6 inches in diameter and $1\frac{1}{4}$ inches

[3] 1 inch = 2.54 cm.—Ed.

FIG. 8. SNOW'S ETHER INHALER OF 1847



This is the last of four different models designed by Snow. The round chamber (B) with spiral path insert is below the lid (E) on the right. The left half of the rectangular box merely serves as a larger reservoir for water which also surrounds B.

A: rectangular box
B: round vapour chamber with spiral path insert
D: tube through which the air enters
E: lid
F: breathing tube
S: section showing path of air in chamber

TABLE I. DATA OBTAINED WITH AN INHALER DESIGNED BY SNOW

Initial water temperature: 62° F.[4]
Respiration pump: 9 l./min.; 27 respirations/min.

Time (min.)	0.5	1	2	4	8
Ether–air mixture (vol. %)	20	15	14	13	12

deep; only a thin layer of ether (4 fluid ounces)[5] covered its bottom, and the spiral partitions attached to the top of the round chamber dipped into the liquid. The need to check the rapid decrease in temperature when considerable quantities of ether evaporate was well appreciated by the inventor; in the rectangular box (A) over 1 litre of water was in contact with both the underside and the wall of the shallow vaporizing chamber.

The opportunity of investigating with modern methods an apparatus one hundred years after its invention does not often present itself, and the data given in Table I may have an added historical interest apart from revealing some of the characteristic properties and faults of such simple inhalers.

In these measurements no further dilution with air was performed, while in the actual inhaler this was carried out by a side-opening on the face mask. Nevertheless, the high concentration at the beginning of the experiment is quite characteristic for such an inhaler. (Until recently many Boyle-type vaporizing bottles had a similar objectionable peak: the unsuitably designed control tap suddenly admits a large proportion of the anaesthetic gases into the ether bottle while the control lever is only gradually advanced.) Although the water temperature scarcely alters in the course of this short experiment, there is a noticeable fall in concentration because the heat transfer from the water to the liquid ether is not perfect. Nevertheless, a comparison with a standard glass Boyle-type bottle shows up Snow's invention in a favourable light (see Table II).

[4] t° F. = $\frac{9}{5}$ (t − 32)° C.—Ed.
[5] 1 fluid ounce = 28.4 ml.—Ed.

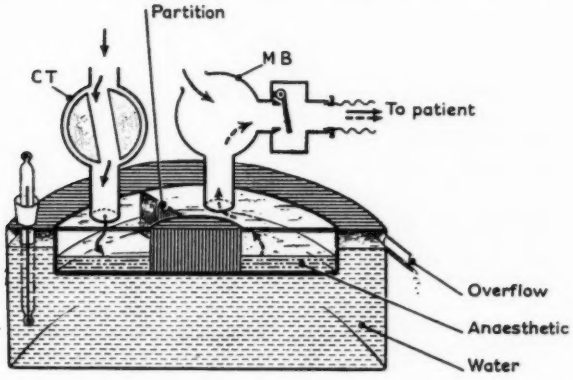
TABLE II. DATA OBTAINED WITH STANDARD GLASS BOYLE-TYPE BOTTLE

Boyle-type ether bottle, 8 cm. diam., filled with 6 fl. oz. ether
Initial temperature: 20° C.
Continuous oxygen flow: 8 l./min.

Time (min.)	0.5	1	2	3	4	6	8
Ether–air mixture (vol. %)	18	15.5	14	12.5	11.5	10.0	9.0

In common with numerous other inhalers designed since, Snow's apparatus showed the following variabilities: the output depended on the temperature and the amount of liquid ether present; it also altered considerably when the inhaler was inadvertently agitated.

FIG. 9. SCHEMATIC SECTION OF LEVY'S CHLOROFORM INHALER OF 1905, SHOWN IN PLATE I, FIG. A



The shallow vaporizing compartment is ring-shaped, and a partition forces the entering air stream to circle over the surface to the outlet, where it is mixed with air entering through an orifice in the spherical mixing box. The filler for the warm water entering low at the bottom of the water tank is not shown. The air stream mixed with chloroform vapour leaving the vaporizing compartment is indicated by the broken arrows.

CT: control tap; MB: mixing box

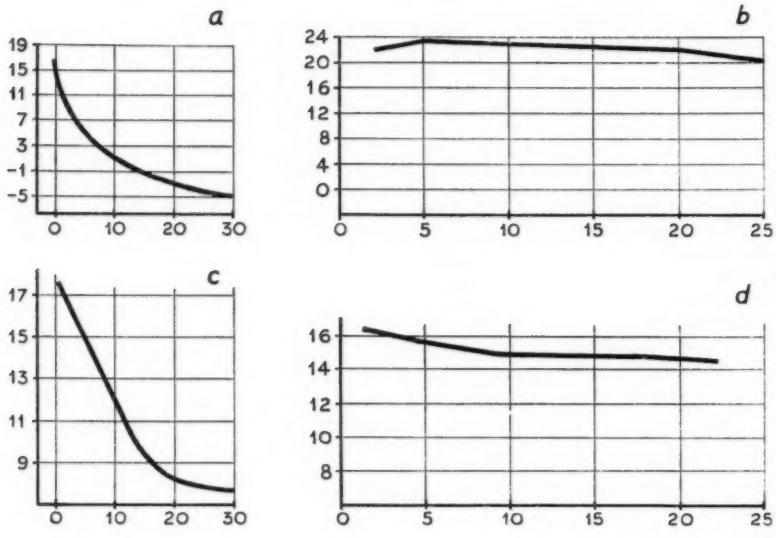
4. Levy's Quantitative Chloroform Apparatus

For about half a century following Snow's death, interest in quantitative anaesthetic inhalers flagged and no progress was made in this direction. In certain respects the years 1904–05 represent a landmark with regard to scientific design of anaesthetic equipment and to detailed information about a commercial apparatus. During those years Levy (1904) published some of the many measurements he had carried out on two types of chloroform inhaler designed by him. As future author of a well-known treatise on chloroform anaesthesia (Levy, 1922), he was convinced that the majority of accidents with this agent were related to excessive and erratic dosage. His views received some confirmation during clinical work with the Epstein, Suffolk, Oxford (ESO) inhalers (Macintosh & Mushin, 1945) used by air-borne troops during the Second World War, and more particularly by the work of Waters and associates on the re-evaluation of chloroform (Waters, 1951). In this connexion it is somewhat surprising to note that the latter authors do not mention the use of any quantitative inhalers for their clinical studies.

Introduction of Manual Thermo-Compensation

Levy introduced the principle of manual thermo-compensation into anaesthetic devices; this term merely signifies that notice is taken of the varying temperatures of the liquid anaesthetic, due either to cooling by evaporation or to ambient temperature changes. A calibrated control tap is shown to the left above the shallow vaporizing compartment (fig. 9). It has the dual function of adjusting the concentration and of compensating for the effects of temperature changes of the liquid chloroform, by altering the proportion of inspired air passing through the vapour chamber. The greater portion of the inspired air enters directly through the hole in the

FIG. 10. FALL IN TEMPERATURE OF LIQUID ETHER, AND DECREASE OF VAPOUR CONCENTRATION IN OUTPUT, WHEN COMPARABLE GAS FLOWS ARE PASSED THROUGH A BOYLE-TYPE VAPORIZING BOTTLE AND A CONSTANT-TEMPERATURE TYPE OF INHALER



Abscissae: time (min.)

Ordinates:

a: temperature of liquid ether in Boyle-type vaporizing bottle (°C.)

b: temperature of liquid ether in constant-temperature type of inhaler (°C.)

c: ether concentration in oxygen stream leaving Boyle-type vaporizing bottle (vol. %)

d: ether concentration in air leaving constant-temperature type of inhaler (vol. %)

spherical mixing chamber adjoining the non-return flap valve on the right. The thermometer records the temperature of the warm water in the jacket surrounding the shallow vaporizing chamber which contains about 2 fl. oz. of liquid chloroform. When the temperature falls, the concentration would decrease if the control tap were not adjusted; a pointer connected to it (Plate I, fig. A) is marked with a temperature scale and moves over a dial covered with lines indicating vapour concentration (the two form a kind of simple nomograph). The effect of setting the pointer to a lower temperature mark is an increased opening of the tap, and a larger proportion of air is passed through the vapour compartment.

5. Ratio of Flow-Rate to Concentration Characteristic of the Vaporizing Chamber

In discussing the performance of an anaesthetic inhaler, the term "characteristic" is convenient to describe the change of concentration with varying volumes of gases passing through the apparatus in one minute while the respiratory rate is kept constant. Levy (1905) determined the characteristic of the vaporizing compartment alone by occluding the diluting hole in the mixing chamber, keeping the regulating tap wide open and the water bath at a constant temperature of 20° C. A bellows pump supplied an intermittent flow of 30 breaths/min., with flow-rates ranging from 2 to 18 l./min. Table III gives an abstract of the original results together with a re-determination by modern methods some fifty years later. The agreement is quite satisfactory considering the different experimental methods of sampling and analysis: the original concentrations were determined gravimetrically, while the "repeats" were done by gas refractometry (Cowan, Epstein & Suffolk, 1942).

In order to supply concentrations in the clinical range from 1 to 4%, only part of the inspired volume is passed through the vapour compartment, and is then mixed with pure air drawn in through the diluting hole in the round mixing chamber; the duct for the diluting air in modern inhalers is often known as "air by-pass".

Assume that the patient inhales a certain concentration for a given respiratory minute volume; if he now increases his inspiration, a greater volume will flow through both the vapour compartment and the air by-pass. For an ideal inhaler the two portions of the total flow are in such a ratio that the concentration of the inhaled mixture remains constant. If the characteristic of the vapour compartment had been a horizontal line (i.e., no change in concentration for varying minute volumes), the ratio of the two flow parts would have to remain constant. For instance, the flow through the vapour chamber would be 2 (or 2.5) l./min. and through the air by-pass 6 (or 7.5) l./min. for respiratory minute volumes of 8 (or 10) litres. The actual vapour compartment has, however, a falling characteristic, and the fraction of the total volume passing through the vapour compartment would have to rise with increasing respiratory volumes. An attempt was made to ensure this by having an orifice in the air by-pass. With increasing flow-rate, the resistance of an orifice rises more rapidly than that of the more tubular ducts in the vapour compartment, so that the proportion of diluting air decreases with increasing minute volume. Despite the soundness of Levy's reasoning, recent tests showed that his apparatus gave a decrease of inhaled concentration with increasing minute volume.

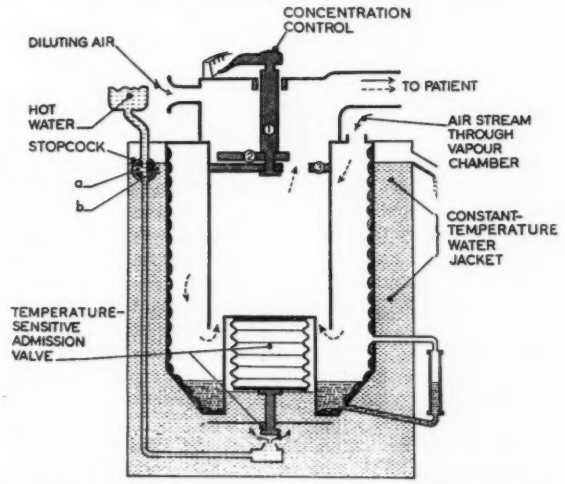
TABLE III. CHARACTERISTIC OF LEVY'S 1905 INHALER: COMPARISON OF ORIGINAL WITH RECENT MEASUREMENTS

Minute vol. (l.)	2	4	6	10	15
Chloroform (vol. %)					
original results	7.6	6.6	5.8	4.9	4.0
recent results	9.7	8.8	7.9	6.2	5.3

6. Inhalers with the Anaesthetic Liquid Kept at Constant Temperature

During the next three decades numerous vaporizing bottles and inhalers were described and used, but none could pretend to supply quantitatively known vapour concentrations under varying conditions of respiration, temperature and other factors. The obvious method of securing a constant vapour strength independent of ambient temperature and of heat loss due to evaporation is to maintain the liquid anaesthetic at constant temperature. Clinical inhalers must be portable and independent of an electric supply; the Oxford Vaporiser (Macintosh & Mendelssohn, 1941) designed at the beginning of the Second World War utilized hot water as heat source. Maintenance of constant ether temperature was ensured by interposing a chemical thermostat between ether and the hot water with its variable temperature. In order to keep the temperature of the anaesthetic constant, but below its boiling point, the thermostat, based on the melting point of a crystalline substance, kept the temperature at 28° C., i.e., 7° C. below the boiling point of ether. The temperature of the liquid ether, and the concentration taken up by an intermittent air flow passed through such a constant-temperature type of inhaler (right half of fig. 10), remain constant for a long time (Epstein & Pask, 1941). For comparison (left half of fig. 10), the fast decrease in temperature and concentration is shown when a flow of oxygen is passed through a Boyle-type vaporizing bottle; these measurements were carried out in 1940–41 when little was known about the performance of such apparatus. By keeping the ether temperature constant at 25–28° C., it is possible to obtain concentrations as high as 25–30% without appreciable fluctuations for varying depth of respiration and during prolonged use.

FIG. 11. ETHER INHALER WITH CONSTANT-TEMPERATURE WATER JACKET (1950)



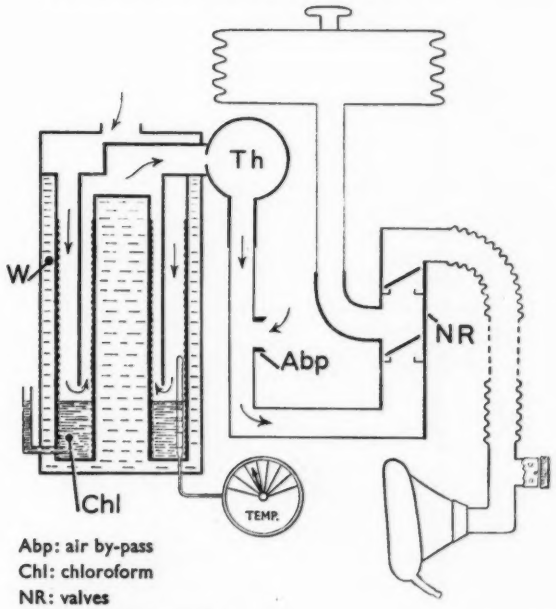
With the stopcock set to (b), the jacket can be quickly filled with water of approximately correct temperature at the beginning of a session; it is then set to position (a), in which the hot water from the tank can flow into the jacket only when the admission valve at the bottom opens.
Shaft (1) carries an eccentric disk (2) which is in close contact with partition (3). For any setting of the concentration control below maximum, the disk partly occludes the hole in partition (3).

The use of a chemical thermostat like that in the Oxford Vaporiser introduces technical complications, and in another inhaler an automatic, hot-water thermostat was used instead (fig. 11). The hot water from a small reservoir mixes with the cooler, constant-temperature water in the jacket surrounding the vapour compartment only if the admission valve at the lower end of the feed pipe opens. The valve opens when the temperature-sensitive metal bellows above it cools below the pre-set thermostat temperature and contracts. It is possible to get a quick response of the valve to suddenly increased evaporation, whenever the control (1, 2) is turned to administer high ether concentrations to the patient. The inhaler was capable of delivering concentrations up to 30% without appreciable fluctuations for a wide range of minute volumes.

7. Chloroform Inhaler without External Source of Heat

The principle of providing a large evaporating area with relatively small free liquid surface was applied in the design of the ESO chloroform inhaler (Epstein, 1955) built for use by air-borne troops in 1942–43 (fig. 12). The part of the inspired volume passing through the vapour compartment has to travel a tortuous path along the walls covered with

FIG. 12. SIMPLIFIED SECTION OF EPSTEIN, SUFFOLK, OXFORD (ESO) CHLOROFORM INHALER, DESIGNED FOR AIR-BORNE TROOPS IN 1943, WITH MANUAL THERMO-COMPENSATOR

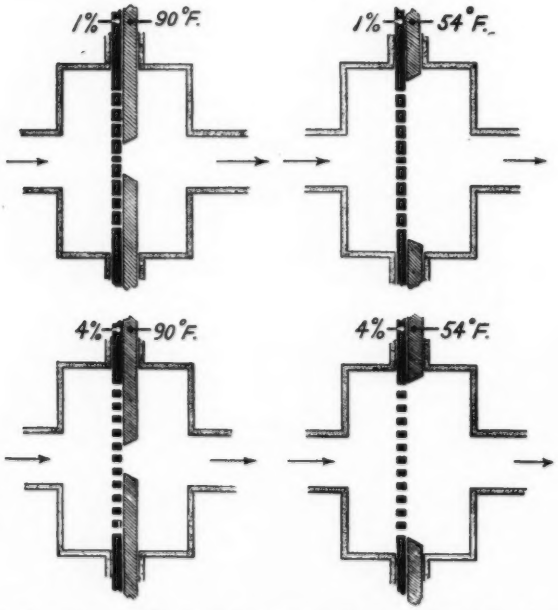


Abp: air by-pass
Chl: chloroform
NR: valves
Th: thermo-compensator
W: jacket and central compartment permanently filled with water

The valves ensure that the concertina bellows can be used for artificial ventilation, and act also as non-return valves during spontaneous breathing. As many plastics tend to warp in chloroform vapour, the actual valves consist of thin, rectangular metal strips with one curled-over edge; the latter is loosely held by retainers forming a kind of free floating hinge.

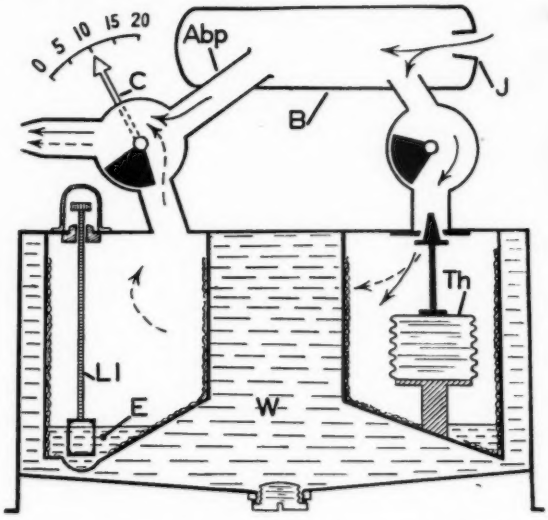
wicks which dip into the liquid (Chl); a jacket and central compartment (W) permanently filled with water act as heat buffer and keep the wick-covered walls at even (not constant) temperature. It is possible to obtain concentrations up to 4% without resorting to the use of warm water as was necessary in Levy's inhaler. The combined concentration control and manual temperature compensator (Th) incorporated two dials. In the schematic representation (fig. 13) the concentration control is shown as the black partition on the left, perforated by numerous holes, and the temperature compensator as the shaded partition on the right, with one orifice of different size for each of the temperature ranges into which the thermometer dial was divided (see Plate I, fig. B). If, for example, the concentration control is set to 1% and the temperature lies in the highest range (90° F.), only a few of the "1%" holes are freed by the small orifice in the compensator partition. If the temperature is in the lowest range (54° F.), the compensator (temperature dial in Plate I, fig. B) must be adjusted appropriately, and a large orifice in the shaded partition allows free passage of air through all the holes of the "1%" position. The mixture travelling downward from the thermo-compensator is diluted at Abp with fresh air; the concentrations indicated on the dial are, of course, those finally inhaled by the patient.

FIG. 13. SCHEME OF COMBINED CONCENTRATION CONTROL (BLACK) AND MANUALLY OPERATED THERMO-COMPENSATOR (SHADED) OF THE ESO INHALER



In the two upper diagrams the concentration control is set to a low value, and in the two lower to a high value as shown by holes of different sizes. The temperature compensator operates in steps, and the illustrated settings are for the highest and lowest temperature range. In the actual inhaler the controls are concentric cylinders made from light alloy; the concentration rotor has numerous graded holes, and the temperature rotor has rectangular windows of various sizes. No lubricant is used between the cylinders as this would tend to block the holes.

FIG. 14. SCHEME OF PROTOTYPE OF EPSTEIN MACINTOSH, OXFORD (EMO) INHALER FOR ANAESTHESIA (1952)



The thermo-compensator contracts and increases the opening in the orifice above it when the ether cools. The water need not be changed; it acts as a heat buffer to reduce the rate of any temperature changes.

Abp: air by-pass
B: distribution box
C: concentration control
E: ether (or other volatile anaesthetic)
J: entry to distribution box
LI: indicator of level
Th: thermo-compensator
W: jacket and central compartment permanently filled with water

8. Automatic Thermo-Compensation in Anaesthetic Inhalers

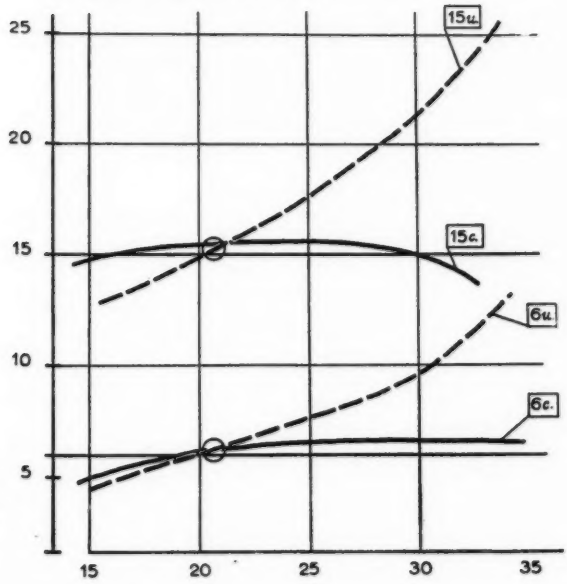
The increasing use of muscle relaxants during the past decade, and the correspondingly lower ether concentrations required in conjunction with them, has made possible the design of quantitative inhalers without any special heat source. The scheme of a model designed in 1952 (fig. 14) shows an automatic thermo-compensator (Th) in the vapour compartment. When the ether temperature falls, the compensator increases the size of the opening above it, and a greater proportion of the inspired air passes through the vapour chamber, while the concentration control (C) remains unaltered. Of course, adequate compensation is possible only within a limited temperature range (16–33° C.), because the warmth of the surroundings is the final heat source. A jacket and central compartment (W) permanently filled with water provide a high heat capacity and prevent any rapid drop in ether temperature even if the control (C) is turned to deliver high concentrations. The diluting air passes here through a tubular by-pass (Abp), as the "characteristic" of this inhaler requires an increasing proportion of diluting air when the minute volume rises, in order to maintain a constant concentration in the inspired mixture.

Some Performance Data of the EMO Inhaler

The effect of an automatic temperature compensator in regulating the ether output for varying ambient temperatures is best shown by a comparison with a similar inhaler in which the compensator is intentionally immobilized. The measurements (fig. 15) were made with production models of the Epstein, Macintosh, Oxford (EMO) inhaler (Epstein & Macintosh, 1956). The concentration pointer was kept at fixed positions ("6" and "15"), and the room temperature varied between 14° C. and 34° C. In one of the inhalers the thermo-compensator was kept locked at its 20° C. position, and the resultant concentrations (u : uncontrolled) are shown by broken lines, while those from the inhaler working normally (c : controlled) are shown by solid lines.

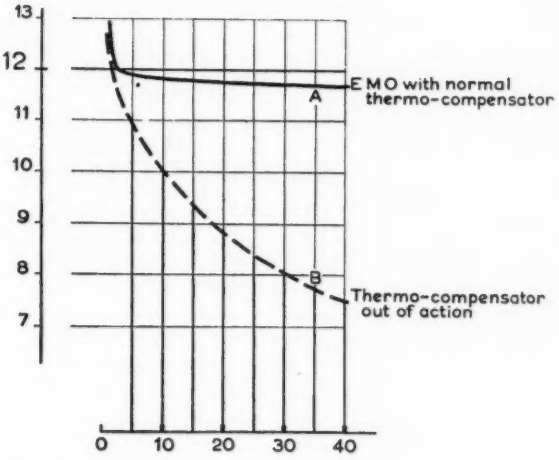
The thermo-compensator also has a significantly steadying effect on the concentrations even if the temperature of the surroundings remains constant (fig. 16). The flow in these experiments, as also in those of fig. 15, was produced by a respiration pump set to a minute volume of 9 litres and a respiratory rate of 22/min. Curve B shows clearly the rapid decrease in concentration during a prolonged run if the compensator is intentionally put out of action. The effect of varying respiratory volumes and rates on the concentration in the output is reduced by appropriate construction of air

FIG. 15. EFFICACY OF THERMO-COMPENSATOR IN MAINTAINING CONSTANT OUTPUT CONCENTRATIONS (FROM EMO INHALER) FOR VARYING AMBIENT TEMPERATURES



Abscissae: temperature (°C.)
Ordinates: concentrations of ether delivered (vol. %)
Solid-line curves: concentrations, with the thermo-compensator in use (c: controlled)
Broken-line curves: concentrations, with thermo-compensator intentionally put out of action (u: uncontrolled)
(Concentration control settings at "6" and "15")

FIG. 16. EFFECT OF THERMO-COMPENSATOR IN CONTROLLING THE OUTPUT FROM EMO INHALER DURING PROLONGED DELIVERY OF HIGH CONCENTRATIONS



Abscissae: time (min.)
Ordinates: concentration of ether delivered (vol. %)
Solid-line curve (A): concentration with thermo-compensator in normal operation
Broken-line curve (B): concentration with thermo-compensator kept fixed in the position corresponding to the initial temperature of the experiment
(Concentration control set at "12"; respiration pump 9 l./min., 22 resp./min.)

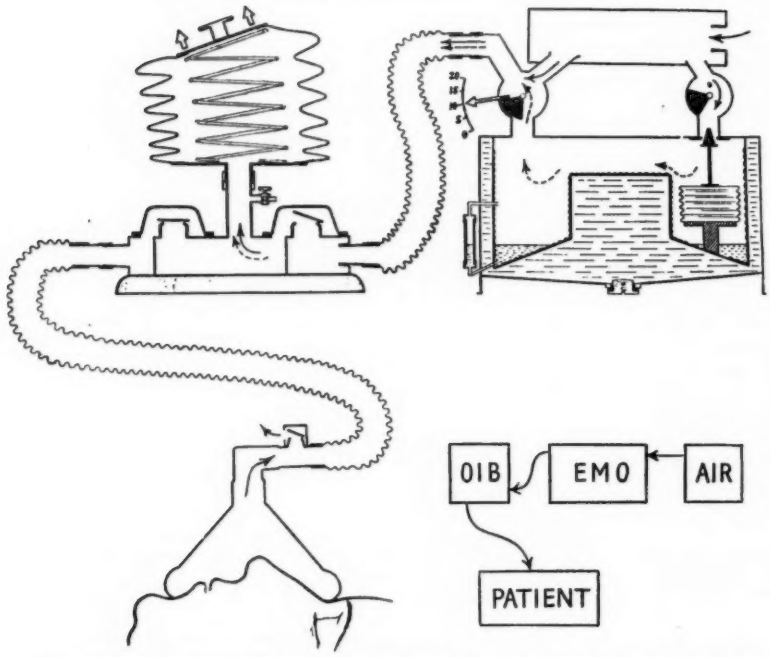
by-pass and vapour-chamber ducts, and also by the interposition of the bellows unit (Oxford Inflating Bellows) (OIB) (Macintosh, 1953) between inhaler and patient (fig. 17). During the expiratory phase illustrated, the slight expansion of the bellows (see white arrows) returning to their resting position draws in some fresh mixture from the inhaler; if the subsequent inspiration should be rather abrupt, some of the tidal volume is taken from the bellows, the remainder is drawn from the inhaler. It is possible to keep within fairly close limits any concentration for a wide range of minute volumes and respiratory rates (fig. 18). The control was set near the middle ("8") of the concentration scale of the inhaler; the flows were produced by a pump imitating a human respiratory pattern, although the output from an EMO inhaler is nearly the same if a sine-wave pump is used instead. Similar inhalers have been constructed for use with Fluothane and other anaesthetics.

The entry (J) to the distribution box (B) in fig. 14 makes it possible to admit anaesthetic gases and to use the apparatus as a vaporizing bottle. Although constructed for intermittent flow, the concentrations delivered in this arrangement are reasonably accurate for continuous flow-rates above 8 l./min.

9. Other Aspects of Inhaler Design

Although only problems relating to vaporization can be discussed here, the breathing resistance is at least of equal

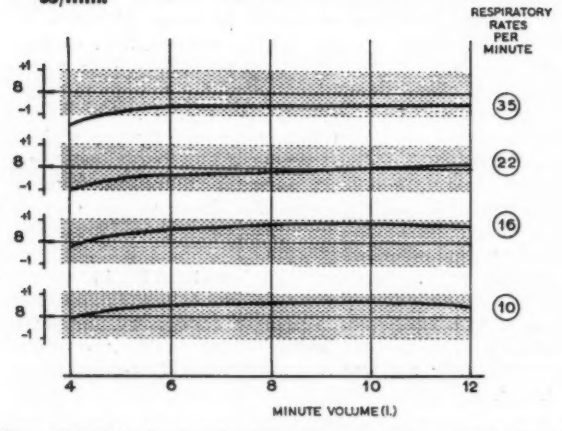
FIG. 17. EPSTEIN, MACINTOSH, OXFORD (EMO) INHALER WITH OXFORD INFLATING BELLOWS (OIB) UNIT ARRANGED FOR ETHER AND AIR INHALATION



When used as a plenum apparatus with anaesthetic gases, the OIB is moved to the right and connected to the inlet of the inhaler. The gases are fed into the entry to the OIB itself which acts as a reservoir and also indicates when the flow-rate is either too high or too low. The corresponding block diagram would be:

PATIENT ← EMO ← OIB ← ANAESTHETIC GASES

FIG. 18. CURVES FOR EMO INHALER INDICATING HOW FAR THE CONCENTRATION REMAINS CONSTANT FOR A RANGE OF MINUTE VOLUMES UP TO 12 l./min. AND RESPIRATORY RATES UP TO 35/min.



The width of the bands is 1 vol.% either side of the nominal value of the concentration control setting (8).

(EMO inhaler operated by respiration pump; ambient temperature: 24° C.)

importance. In fact, the laudable tendency to diminish the breathing resistance of anaesthetic apparatus has complicated the design of quantitative inhalers, because the use of wide ducts makes it difficult to influence the inhaler "characteristic". Neither are inhalers for analgesia considered here (Epstein & Macintosh, 1949). It should be recalled, however, that the present interest in quantitative vaporizing devices was heightened by the extensive work on apparatus for obstetric analgesia with Trilene; this was carried out by various committees, research establishments and commercial firms between 1946 and 1954. Quite recently the makers of one of the Trilene inhalers for use by midwives have developed a quantitative vaporizing bottle (Fluotec) for evaporating Fluothane with anaesthetic gases (MacKay, 1957).

ACKNOWLEDGEMENT

Prototypes of the inhalers developed at Oxford were constructed in collaboration with Mr Richard Salt, Chief Technician to the Nuffield Department of Anaesthetics.

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PRINCIPLES OF INHALERS FOR VOLATILE ANAESTHETICS

(FIGS. A, B)

H. G. Epstein

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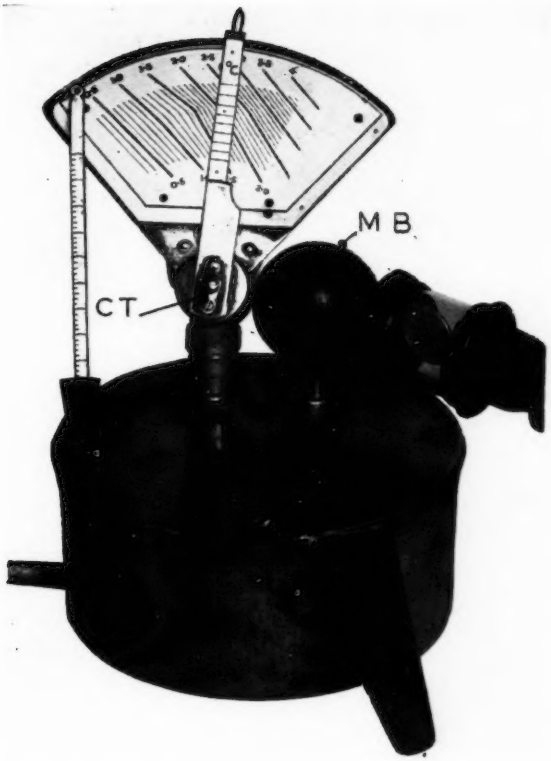

FIG. A. "REGULATING CHLOROFORM INHALER" DESIGNED BY LEVY IN 1905

This is the second of the two models designed by Levy. The non-return valve visible in the glass window on the right of the apparatus was metal-backed, a flexible flap acting as hinge. The inhaler was filled with chloroform through a triangular opening just visible near the front edge of the inhaler top. The openings for control tap and air by-pass are behind the control tap and the spherical mixing box

CT: control tap; MB: mixing box

FIG. B. PRODUCTION MODEL OF ESO CHLOROFORM INHALER

In order to avoid sudden peaks of concentration, a locking device ensures that the temperature compensator can be moved only when the concentration is set at 0%. The upper valve (see NR fig. 12) is visible as a white spot in the plastic window above the metal thermometer on the base of the apparatus. The combined expiratory valve and face mask connector is a light alloy casting. For inflation the top is rotated; this seals the side openings in the valve house without interfering with the mechanism of the expiratory valve

UNEVEN VENTILATION OF THE LUNGS

E. A. Pask

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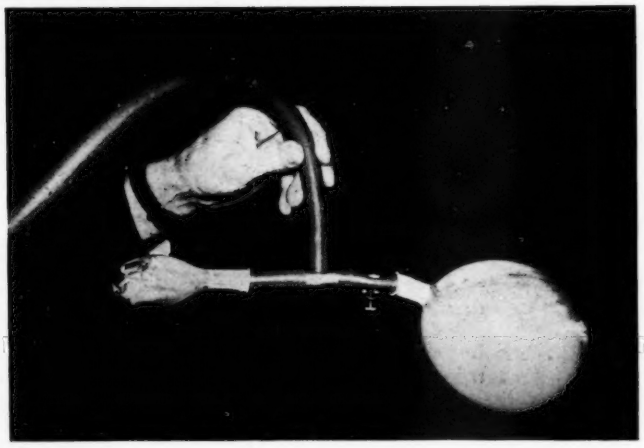
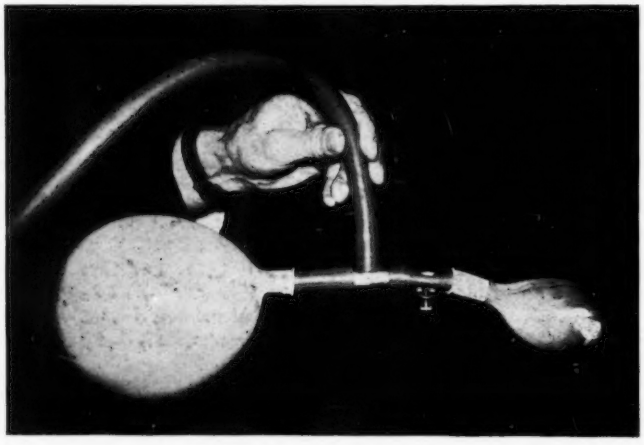
Simple model illustrating the possibility that the relative ventilation of parts of the lung may be altered by such factors as the rate of inflow of air

a: the two balloons are connected to a T-piece. The balloon on the right is highly compliant, but entry of air is restricted by a "viscous" resistance (screw clip). The balloon on the left is more tense and has poor compliance, but the entry tube is unrestricted



b: when the two balloons are inflated slowly, almost all the air enters the compliant balloon



c: when the air flow is rapid, the less compliant balloon receives most of it because the "viscous" resistance hampers rapid entry of air into the right-hand balloon

CARBON-DIOXIDE HOMEOSTASIS IN ANAESTHESIA

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1 Drugs
2 Instruments
3 Artificial respiration
4 Carbon-dioxide storage
5 Hypothermia
References

The control of CO_2 concentration within the body of a healthy man, breathing air, is a complicated business, as yet imperfectly explained. The control of CO_2 movement and concentration within the body of an anaesthetized patient must in many ways be more difficult to elucidate.

The gaseous environment is no longer the fairly constant atmosphere, but is a variable mixture of gases delivered by a machine which has perplexing characteristics of elastic and viscous resistance to air flow as well as providing an instrumental addition to the patient's own dead space. In this sense, even the apparently simple open mask is an "apparatus" and indeed a rather unpredictable one.

Furthermore, the humoral environment is altered by the drugs administered and these may produce secondary relevant effects, for example, bronchomotor or bronchosecretory activity. The surgical operation, the posture on the operating table and the patient's disease all add to the influences which may constrain and confound the physiological processes which usually operate to preserve CO_2 homeostasis.

Anaesthetists sometimes look back upon the "bad old days" when the patient's respiratory needs seem to have been largely ignored. It is, however, well to remind ourselves that Joseph Clover was well aware of the shortcomings of his famous inhaler, and in a letter for the *Lancet* concerning artificial respiration, he wrote:

> I believe the Silvester and Marshall Hall methods and even the intermittent compression of the chest and abdomen would rarely fail if the air passages were free. Our experience with gas and ether and ether and air *breathed over and over again*, show that a very small quantity of air is sufficient to maintain life and to restore the respiratory movements when they become intermittent.

1. Drugs

No doubt in those days the brevity of operations and the ruggedness of human beings prevented the frequent occurrence of trouble. By the time that longer surgical operations became usual, open-mask techniques were popular and light diethyl ether anaesthesia, with its over-all respiratory stimulant activity, was widely accepted.

However, practically all anaesthetic agents, including ether, are now known to depress the response of the central breathing-control mechanisms to CO_2 (Dripps & Dumke, 1943), and in recent years, with the exception of light ether anaesthesia, their over-all depressant action in strictly clinical circumstances (Swerdlow, 1957; Swerdlow & Newman, 1957; Scurr, 1956) has repeatedly been demonstrated.

Diethyl ether seems unique amongst the agents capable of producing full surgical anaesthesia in that, in light or moderate degrees of anaesthesia, the minute volume may be increased. This effect is probably due to a variety of causes, including metabolic acidosis, adrenaline release and peripheral nerve stimulation in the air passages.

The recognition of this central respiratory depressant action of nearly all anaesthetics underlines the importance of abnormal CO_2 concentrations in the inspired air. When the physiological mechanisms are intact, over-ventilation occurs in response to increased inspired CO_2 concentrations, and the change in CO_2 tension in the alveolar air is minimized. When the liveliness of this response is reduced, accumulation of CO_2 readily occurs (Dripps & Severinghaus, 1955). The possibility has been suggested that relaxant agents might exert a central depressant action on respiration, in addition to their neuromuscular action. Animal experiments give little support to this idea in respect of agents of the curare type. Measurements upon anaesthetized patients have failed to demonstrate such an effect with suxamethonium (Davison & Rees, 1957).

2. Instruments

The commonest reason for an increase in inspired CO_2 concentration during anaesthesia is an increase in the respiratory dead space, either within the body or within the apparatus. The former may occur through disease, or because surgical pneumothorax permits pendulum air flow to and from the exposed lung, or perhaps in some degree because of the action of drugs. Bronchodilator drugs may increase the respiratory dead space. The single-breath technique has been used to show that atropine may cause a moderate increase in the anatomical dead space in man (Severinghaus & Stupfel, 1955).

The matter of instrumental dead space becomes particularly important in children, and an increasing realization of the small tidal volumes and vital capacities in small children (Ferris & Smith, 1953; McIlroy & Tomlinson, 1955) has led to many new attempts to reduce the instrumental dead space in children's equipment. There has been a reappraisal of the behaviour of gases flowing through the common types of anaesthetic apparatus, particularly in relation to the rebreathing of vitiated air. This is dealt with in the paper by Mapleson, p. 64 of this Bulletin. It is important to be quite sure what it is that these investigators have attempted to measure. Some have sought to measure a purely physical quantity: for example, whether or not expired gases flow back into the equipment. Some have concerned themselves with the development of what they have regarded as intolerable CO_2 concentrations in certain clinical circumstances. Others have attempted to define flow-rates of fresh gases which, in a particular piece of equipment, may be expected to prevent the inspiration of what are, in their view, undesirable CO_2 concentrations. Such figures, when related only to the machine and not also to such values as the tidal volume and minute volume, cannot have any real physiological significance.

The technique of closed-circuit anaesthesia with absorption of CO_2 by soda lime continues to be used in the United Kingdom, though it is doubtful if the circuit is nowadays often completely closed. Because of the popularity of mixtures of nitrous oxide and oxygen as a means of keeping patients unconscious in the presence of relaxant agents, a generous inflow of mixed gases with some outward leakage is

usual. It is difficult to maintain an effective concentration of nitrous oxide without risk of hypoxia in a completely closed circuit.

Nevertheless, the conditions for best functioning of soda lime have received further attention and, in particular, the importance of correct packing of containers of soda lime has been stressed (Lund, Lund & Erikson, 1957; Robson & Pask, 1954). It is unfortunate that even now the only available standards for soda lime allow variations which may render a product unsatisfactory for anaesthetic purposes, and that no satisfactory test procedure has been evolved to cover the liability of the product to break down into dust.

Viscous resistance to air flow and elastic resistance to air movement in anaesthetic equipment have received a good deal of attention in recent years. Many measurements have been recorded on such equipment as endotracheal tubes and connectors, on respiratory valves and anaesthetic reservoir bags (Mapleson, 1954; Mushin & Mapleson, 1954; Ruben & Hesse, 1956). At least one specification for anaesthetic apparatus (Medical Research Council, 1954) now contains a requirement for resistance to air flow.

The effect of such added resistance upon the efficiency of spontaneous or artificial respiration is still incompletely determined. In conscious men, viscous resistance to breathing results normally in increased inflation of the lungs so that more work is done during inspiration, and sufficient elastic tension is developed in the lungs and chest wall to overcome the resistance to expiration by passive recoil. This reaction occurs for moderate degrees of respiratory resistance and in circumstances where the minute volume is not increased, for any reason, to more than three to four times resting values. The effect of respiratory resistance is not necessarily to reduce minute volume—it may, in fact, increase it somewhat.

Campbell, Howell & Peckett (1957) have recently shown in a small series of cases that during light anaesthesia the same sort of response to respiratory resistance occurs. Much more work is needed to discover the reaction of more deeply anaesthetized patients and those suffering from anoxia, but clinical observation suggests that the response of patients with circulatory insufficiency may be quite different.

3. Artificial Respiration

The advent of relaxants has introduced a new problem in relation to CO_2 homeostasis. When a patient's central breath-regulating mechanism is depressed by anaesthetics, and his peripheral effector mechanism is impaired by neuromuscular blockade, it is not to be expected that the homeostatic mechanisms related to CO_2 can remain effective.

It is perhaps unfortunate that in the early days of relaxants they were often used in small or moderate doses. Usually, some respiratory movement persisted and, because it did, the anaesthetists sometimes refrained from giving artificial respiration.

The figures in *A study of deaths associated with anaesthesia and surgery* by Beecher & Todd (1956) suggest that, amongst those patients who were given relaxant drugs, a considerable proportion did not receive artificial respiration or assisted respiration. Of the total number of patients who were given anaesthetics by inhalation, about 14,000 received assisted or artificial respiration. However, 44,000 patients received relaxants and it seems likely that most of these also fall into the category of anaesthesia by inhalation. Thus about 30,000 patients seem to have been given relaxants without receiving artificial respiration. It is well known that in this particular investigation the use of relaxants seemed often to be associated with an unfavourable outcome, for one out of every 420 patients receiving relaxants died.

It is now generally recognized that, in the presence of respiratory embarrassment of this kind, artificial respiration is necessary and this has placed upon anaesthetists the responsibility of assuring themselves that the available methods of artificial respiration can be effective. It is interesting to reflect that twenty years ago, at any particular moment, there might in the United Kingdom have been a few score people whose lives were depending entirely upon artificial respiration. These would have been emergency cases of drowning and of other forms of asphyxiation, and a few patients with chronic respiratory paralysis. Nowadays, at any moment, there must be many hundreds of patients whose lives depend entirely upon artificial respiration, and most of them are to be found in operating rooms. So common is the manoeuvre that it comes almost to be forgotten: but we should remind ourselves that artificial respiration is now the common factor in almost all relaxant techniques and until we know a great deal more about its effects it will be difficult to identify clearly the pharmacological actions of the individual relaxants themselves in clinical practice.

In 1951 a disquieting observation emerged from the exhaustive examination of methods of *manual* artificial respiration (Gordon, Prec, Wedell, Sadove, Raymon, Nelson & Ivy, 1951) which was then carried out by a group of universities in the United States of America. It was that, using certain methods, though an apparently adequate volume of air passed into the lungs each minute, the oxygen saturation of the blood remained abnormally low. Thus the possibility was underlined that the artificial introduction of air into the lungs might not contribute to gas exchange with the blood in the same degree as would an equivalent volume of spontaneous ventilation. These workers suggested that the reason might be that the ventilation of the lungs was uneven so that air and blood did not properly meet. Such inefficiency could be alleviated in respect of anoxia by increasing the oxygen concentration in the inspired air, but it would be a more serious matter in relation to the removal of CO_2. Since that time, a good deal of work has been done upon the topic but a great deal remains to be done.

The "inefficiency" of artificial respiration, if it exists, may always be present in normal men; it may be present only when the surgical conditions are abnormal (for example in the presence of pneumothorax) or when an unusual operating position is needed; or it may be of concern only when the patient's lungs are abnormal. This last possibility would not mean that the phenomenon was necessarily unusual.

Earlier papers (Beecher & Murphy, 1950; Taylor & Roos, 1950) suggested that a given minute volume of artificial respiration would not so effectively remove CO_2 from the body as an equivalent volume of spontaneous ventilation, particularly when, in the presence of surgical pneumothorax, artificial respiration has perforce to be used. More recent work (Martin, Cline & Marshall, 1953; Stead, Martin & Jensen, 1953) has confirmed the risk of respiratory acidosis during thoracic surgery of long duration. When the chest wall was intact, Lucas & Milne (1953), who estimated arterial pCO_2 indirectly, considered that respiratory acidosis developed in some patients despite minute volumes of 10 l./min. The careful work of Eastwood & Harbord (1954), who measured end-expiratory pCO_2, suggests that the artificial

minute volume may need to be greater than that of equally effective spontaneous breathing. On the other hand, Scurr (1956), who measured the pCO_2 in a rebreathed "equilibrium" gas sample, was able to relate these readings to ventilation volumes and to include readings from spontaneous and artificial breathing upon substantially the same curve. This suggests that, in his trial, equal volumes of the two types of breathing may have been equally effective in the removal of CO_2. Dobkin and his colleagues conclude, in general, that artificial respiration can secure adequate elimination of CO_2, though they suggest that minute volumes may have to be generous (Dobkin, Hubay, Mendelsohn & Hingson, 1956; Dobkin & Wyant, 1956). Interesting preliminary publications by Nunn and Pincock have so far dealt mainly with relevant techniques of measurement (Nunn, 1956; Nunn & Pincock, 1957).

If this inefficiency of artificial respiration exists, it may be due to many factors. A deleterious effect on pulmonary circulation has been suggested: the differing viscous and elastic resistances which govern entry of air into the various parts of the lungs might give rise to an abnormal distribution of air, when artificial respiration takes the place of spontaneous breathing. The abnormality of the distribution might vary with variations in the rate of inflow of air during inspiration (Otis, McKerrow, Bartlett, Mead, McIlroy, Selverstone & Radford, 1956). Plate II, fig. A illustrates this point.

The functioning dead space might alter during artificial respiration because of changes in the calibre of the bronchial tree (Severinghaus & Stupfel, 1955); there may be "trapping" of air in parts of the lung because bronchi are occluded by pressure. Changes in the systemic circulation may be involved for this controls the rate at which CO_2 is brought to the lungs for excretion. All these possibilities have been suggested though it must be admitted that, on theoretical grounds, several of them seem unlikely. Further progress may perhaps be possible by attempting to apply tests specifically designed to reveal one or other defect.

It seems to be clear that, in the presence of surgical pneumothorax, some such "inefficiency" of artificial respiration is to be allowed for, and more than normal tidal volumes must be induced. However, in a sense, the situation is less serious here as artificial respiration is indispensable, and the problem is to find the best way of achieving it. During abdominal surgery, artificial respiration *could* be avoided, though to do so would deny to the patient many advantages. Therefore, the establishment or otherwise of this suggested inefficiency of artificial respiration is of more importance in this situation. In my opinion many more careful clinical measurements are needed, of as simple a character as possible, upon a variety of patients. In these abnormal conditions, measurements of such quantities as "alveolar CO_2 tension" are indeed difficult. Not only are there technical problems, but what constitutes "alveolar air" is difficult to define. The proof of respiratory acidosis by estimation of the CO_2 tension of the arterial blood requires extreme care in the handling of samples and great precision in the estimation of plasma pH and CO_2 content. Perhaps the development of more direct methods of estimating pCO_2 may assist this approach.

4. Carbon-Dioxide Storage

Meantime, anaesthetists would do well to consider the conception of the carbon-dioxide balance-sheet utilized by Shepard (1955). The minute output of CO_2 is a quantity which can be measured quite reliably, and records of this quantity related to the minute volume of respiration would give some indication of whether CO_2 accretion or loss were occurring. The extent of the body stores of CO_2 has recently received a good deal of attention. These considerable stores form a reservoir which can maintain the plasma CO_2 tension when the gas is being lost from the body and can buffer the effects of CO_2 accretion. Their magnitude and their availability are not yet precisely known in man, but figures from animals suggest that there may be some 40 or more litres of CO_2 in the body (Freeman & Fenn, 1953), though much of it is in bone and may not be available for ready exchange with the blood (Farhi & Rahn, 1955).

Anaesthetists often assume that excessive ventilation of the lungs is much less dangerous than under-ventilation, and, if only because hyperventilation is not associated with oxygen want, this assumption is almost certainly true. Nevertheless, it has recently been suggested (Clutton-Brock, 1957) that respiratory alkalosis in anaesthesia might lead to damage to the central nervous system by initiating intracranial vasoconstriction and anoxia. Many anaesthetists have sensed that "excessive" ventilation with mixtures of nitrous oxide and oxygen seems to confer upon them greater anaesthetic properties than would be expected. There may, of course, be many explanations for this impression and there is no particular evidence to recommend the proposal that respiratory alkalosis is making a contribution to the depth of anaesthesia.

It has long been known that violent voluntary over-ventilation can so lower the plasma pCO_2 and raise the pH that unconsciousness occurs with marked electroencephalographic changes. However, in these circumstances, the ventilation may be of the order of 100–150 l./min., and the removal of CO_2 from the body is such a rapid process that there can be little time for transfer of the gas from the body-tissue stores and certainly not from bone. The plasma level will be very far from equilibrium with the total body CO_2. Amongst others, Lucas & Milne (1953) have pointed out that artificial respiration during anaesthesia, by manual compression of the rebreathing bag, is unlikely to produce ventilation in excess of 14 l./min., which is a very different figure. Such ventilation will lead to a fall in the alveolar CO_2 tension so that the air extracted will be less rich in CO_2 and the ability of the over-ventilation to remove the gas from the body will be less than appears at first sight. Some preliminary measurements made in my Department suggest that if, for example, the end-expiratory CO_2 tension is remaining constant in a particular patient with a passive ventilation of 5 l./min., deliberately doubling this ventilation over a period of 30 minutes would result in the extraction of an "excess" of CO_2 of the order of 800 ml. This loss would correspond with a decrease of 2–4% (v/v) in the plasma CO_2 content and a rise of 0.01–0.02 pH units, depending upon the extent to which the pCO_2 in the various body compartments remains in equilibrium. In other words, the extent of over-ventilation which is possible during anaesthesia by artificial respiration conventionally performed is limited, so that extreme plasma CO_2 depletion is unlikely, provided that equilibrium between the air in the lungs, the blood and the tissue stores is maintained. How badly this equilibrium may be disturbed under anaesthesia, we do not yet know, but we must remember that disturbances of circulation, as well as respiratory upset, could distort it.

During under-ventilation, the only possibility of maintaining the CO_2 output must depend upon a rising alveolar

concentration of the gas and this will certainly correspond with an increase in the plasma CO_2 tension. Thus, in underventilation we may expect soon to see the effects of hypercapnia upon cardiac action (Johnstone, 1950; Rollason & Parkes, 1957), and upon the central nervous system (Scurr, 1954; Pask, 1955). The question must then arise: "What exactly are the damaging effects of high plasma CO_2 tensions upon the *human* body?" Recent work suggests that only limited help may come from experiments upon animals; for many species, and particularly small animals, seem able to survive CO_2 concentrations which would probably be fatal to man. For example, dogs can tolerate the inhalation of 30% CO_2 for two hours. Non-fatal cardiac effects are seen, perhaps due to ionic shifts between the intracellular and extracellular compartments. In particular, the plasma potassium level increases notably. When the inhalation ceases, an "off-effect" with falling cardiac output and diminished peripheral resistance is seen, which somewhat resembles the condition in man which has been described as "cyclopropane shock" (Dripps, 1947). We must, however, remember that these experimental circumstances are probably vastly different from any that occur during normal anaesthesia, though they could no doubt arise from faulty technique.

The procedure of diffusion respiration first described by Hirsch (1905; Holmdahl, 1956) and first named and studied by Draper & Whitehead (1944), is now proving a very useful tool in the study of the effects of the failure to eliminate CO_2. Oxygenation can be maintained for long periods, but the metabolic CO_2 is almost completely retained within the body. Severe respiratory acidosis develops and at a later stage a metabolic acidosis also occurs. At first, cardiac output falls and the peripheral resistance diminishes also, though later the total peripheral resistance seems to rise again somewhat (Holmdahl, 1956).

5. Hypothermia

Finally, some mention must be made of the problems of CO_2 homeostasis in the new field of hypothermia. The problems are extremely difficult from both practical and theoretical aspects, and it is much too soon to attempt any summary. Brewin and others (Brewin, Gould, Nashat & Neil, 1955) have warned against the too easy acceptance of over-simplified views and in several valuable papers have analysed some of the difficulties.

The behaviour of blood when exposed to various CO_2 tensions is clearly of fundamental importance, but the changes in this behaviour when temperature is reduced are not yet fully understood. At lower temperatures the solubility of CO_2 in plasma is increased, and it was sometimes thought that this would lead inevitably to acidosis unless steps were taken to make sure that the pressure of CO_2, to which the blood was exposed in the lungs, was appropriately reduced. However, another change occurs simultaneously as temperature falls. Additional base becomes available for combination with CO_2 so that, in fact, the curve relating pCO_2 and pH is similar at 38° C. and at 26° C., in true plasma. The additional base available to CO_2 results from the decreased ionization of protein at lower temperatures, and the chief protein concerned is apparently haemoglobin. For this reason, the results obtained when separated plasma is used for experiment can be misleading.

The similarity of the pCO_2: pH relationship of true plasma at 38° C. and at 26° C. has been determined in vitro. It is not yet quite certain that the conclusion applies to the behaviour of blood in vivo, nor, of course, must it be taken to mean that the relationship would not be disturbed if metabolic acidosis develops, as it well may do. For anaesthetists it means, however, that one strong argument for attempting to maintain a very low alveolar CO_2 tension during hypothermia has lost validity.

The main lesson to be learnt is that of the need for extraordinary caution in formulating arguments about what *should* happen to CO_2 homeostasis in hypothermia. Brewin *et al.* (1955) draw attention to the fact that the dissociation constant of water varies with temperature so that the "neutrality point" on the pH scale is 7.00 at 25° C., but is 6.80 at 38° C. and 7.50 at 0° C.; an observation which should be sufficient warning against facile theorizing!

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THE MUSCLE RELAXANTS

Recent Developments

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5 Muscle pains
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1. Mode of Action

The chemical theory of normal neuromuscular transmission is now fully established, and on this basis the mode of action of the various relaxants has been determined. D-Tubocurarine is believed to act by passively combining with the protein molecules of the motor end-plate region and thus denying the acetylcholine molecules access to this region. Consequently acetylcholine molecules are formed in a normal manner but are prevented from depolarizing the end-plate region and thus bringing about a contraction. This type of neuromuscular block has been termed " competitive inhibition " or " non-depolarization block ". Amongst its most important clinical characteristics is the finding that anticholinesterase drugs—by permitting the concentration of acetylcholine molecules to build up—can reverse the block if the concentration of D-tubocurarine is not excessive.

Decamethonium and succinyldicholine, on the other hand, are believed to have an affinity for the protein molecules of the end-plate receptor equal to that of acetylcholine. Also, they are both capable of bringing about depolarization, and the resultant firing-off of groups of muscle fibres is seen clinically as fasciculations or twitches throughout the body. Following this brief depolarization, the end-plate region remains in a state of altered sensitivity, so that it requires an end-plate potential several times greater than normal before further depolarization can take place. This type of neuromuscular block is termed " depolarization block ". Clinically it is characterized by the appearance of fasciculations and also by an increase of the block on the injection of an anticholinesterase substance.

2. Mixed Block

There are, however, two other types of neuromuscular block that occur clinically in this region. First, a mixed block arises when drugs of the two main types of relaxant (i.e., depolarizing and non-depolarizing) are used in the same patient. For example, a patient may first be given a dose of succinyldicholine to assist in the passage of an endotracheal tube and then later given D-tubocurarine to produce abdominal relaxation. The result is that some of the motor end-plates are under the influence of a depolarizing drug, whereas others are affected by the non-depolarizing drug. Consequently, a mixed block is present.

The indiscriminate mixing of the two main types of muscle relaxant must be severely deprecated. In the case of succinyldicholine, it is always assumed—sometimes quite erroneously—that the return of activity in the respiratory muscles is a sign of the cessation of neuromuscular blocking activity in the other muscles throughout the body. Following the resumption of respiration after succinyldicholine, some anaesthetists believe it is safe to administer a relaxant of the non-depolarizing type. Each patient, however, is a law unto himself, and the degree of response to the non-depolarizing relaxant (e.g. D-tubocurarine) will depend on the presence or absence of any degree of dual block (see below). Clearly the administration of succinyldicholine to aid in closure of the peritoneum after a long abdominal operation in which D-tubocurarine has been used carries with it the greatest possible risk of prolonged paralysis.

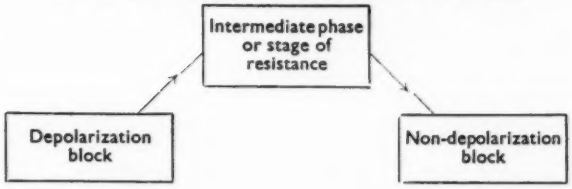
3. Dual Block

This type of block is characterized by a change in the response of the motor end-plate from one of depolarization to one of non-depolarization (Churchill-Davidson & Richardson, 1953). The change in the reverse direction, i.e., from a non-depolarization block to a depolarization block, does not take place; and therefore the alteration in a " dual response " is always in one direction only. It is important to emphasize that it is not the drug itself which alters but rather the manner in which the motor end-plate responds. It occurs normally in certain species of animals (Zaimis, 1953), and was first described in man as occurring in patients with myasthenia gravis: since that time, dual block has been demonstrated in normal subjects (Brennan, 1956).

Theoretically the underlying mechanism is that the molecules of any depolarizing drug first bring about depolarization in the normal manner, but then—in certain circumstances—the protein molecules of the motor end-plate undergo some change which enables them to respond to the same molecules in the way already described for D-tubocurarine. That is to say, they merely adhere to the end-plate region but do not bring about further depolarization—i.e., a non-depolarization block. Under normal conditions this change is a slow process, probably involving some motor units before others, so that for a time some motor units are in a simple depolarization block, others in the intermediate phase, and yet others in the stage of non-depolarization block (see fig. 1).

Animal experiments have shown that the two main types of neuromuscular block, i.e., depolarization and non-depolarization, are antagonistic (Paton, 1956), and this can

FIG. 1. DIAGRAM OF THE DUAL RESPONSE



also be demonstrated in man (Brennan, 1956). For example, if a small dose of D-tubocurarine (5–7 mg.) is given before the injection of succinyldicholine, then the dose of the latter drug required for the abolition of respiratory movement may be several times greater than that normally required. In the case of a dual block (where the motor end-plate response changes from one of depolarization to one of non-depolarization), theoretically there must be a point where the two types of block are antagonistic—i.e., the intermediate or resistant stage. This has not, as yet, been demonstrated in normal subjects, but can be clearly seen in cases of myasthenia gravis in which a similar type of block is believed to occur.

Brennan (1956) has shown very neatly that the action of succinyldicholine changes if repeated doses are given. Thus, of 25 patients given a prolonged infusion of succinyldicholine, 22 showed a dramatic reversal of paralysis on the injection of neostigmine. Similarly, a group of patients were each given a minute dose of gallamine triethiodide after a long infusion of succinyldicholine: the result was an immediate increase in the degree of neuromuscular block. Both these observations suggest that repeated doses of a depolarizing drug in normal subjects lead ultimately to a dual type of neuromuscular block. More recently, a case has been reported in which it is believed that a dual block occurred following a single injection of succinyldicholine (Jowell & Wood-Smith, 1957). Some support is given to this latter finding by the observation that fasciculations are rarely observed on the second or subsequent doses of succinyldicholine.

4. Present Position of Neuromuscular Block in Man

It is no exaggeration to say that the whole conception of neuromuscular block is rapidly undergoing a change as fresh evidence comes to hand. In the light of our past experience, there is ample proof that animal experiment alone is insufficient and that every statement made must be confirmed in man. The present position is represented in a simplified form below (Table I).

TABLE I. TYPES OF NEUROMUSCULAR BLOCK

Depolarization .	Acetylcholine, succinyldicholine and decamethonium
Non-depolarization	D-Tubocurarine chloride, gallamine triethiodide
Mixed block .	In the presence of a combination of depolarizing and non-depolarizing drugs
Dual block . .	Motor end-plate responds first by depolarization and then by non-depolarization

5. Muscle Pains

One of the principal disadvantages of the clinical use of succinyldicholine is the occurrence next day of severe and generalized muscle pains in a large percentage of cases (Churchill-Davidson, 1954 ; Morris & Dunn, 1957). These pains are clearly related to the amount of exercise the patient takes in the few hours immediately after the succinyldicholine has been given. If, therefore, the patient is confined to bed for the first 24 hours, the incidence and degree of the pains are minimal in comparison with those in an out-patient.

This problem of post-operative muscle pain has become so severe in some cases that many anaesthetists have now abandoned the use of this drug. The occurrence of these pains does not appear to be related to the speed or the amount of the injection, and similarly the magnitude of the fasciculations cannot be correlated with the severity of the pains (Morris & Dunn, 1957). It is known, however, that a small dose of a non-depolarizing drug (such as D-tubocurarine) immediately preceding the injection of succinyldicholine will prevent the fasciculations and reduce both the incidence and the severity of these pains. This combination of the two types of relaxant drug, however, is not safe in clinical practice.

Clearly, the ideal substitute for succinyldicholine would be an ultra-short-acting drug of the non-depolarizing variety, but, to date, the pharmacologists have been unable to find a suitable drug of this type.

6. Abnormal Response to the Muscle Relaxants

During the past few years the muscle relaxants have been widely used in clinical surgery and occasionally their duration of action has been noted to be longer than expected. Accounts of " prolonged apnoea " in the literature have been numerous, and the number of possible theories to explain its occurrence has been even greater. Basically these can be divided into two main groups, namely factors acting on the brain and spinal cord—thus preventing respiration centrally—and, on the other hand, those factors acting on the neuromuscular junction and preventing activity by blocking the peripheral mechanism.

a. Central Causes

One of the commonest reasons why a patient fails to respond after a long period of muscle paralysis with a relaxant drug is not connected with the neuromuscular block but with a central depression of the respiratory centre by an analgesic or barbiturate drug. Often pethidine has been given in such cases and plays the principal part in suppressing the onset of respiratory activity, and for this reason is best omitted in any case in which controlled respiration is used. Alterations in the carbon-dioxide content of the blood play an extremely important part in determining the onset of normal respiratory activity. In the presence of muscle paralysis and controlled respiration, the carbon dioxide rarely remains at normal levels. Usually hyperventilation with carbon-dioxide absorption is practised and the patient undergoes a mild alkalosis; at the end of the operation, cessation of carbon-dioxide absorption and slowing down of the respiratory rate enable the carbon-dioxide level of the blood to rise again and so the respiratory centre again becomes excited.

If the degree of overbreathing has been excessive, then it may be some time before the respiratory centre resumes activity again, in which case hyperventilation must be considered as a cause of prolonged apnoea.

In some cases under controlled respiration the anaesthetist fails to ventilate the lungs adequately, and so the carbon-dioxide level of the blood rises and an acidosis results, due to hypoventilation. This brings about an insidious poisoning of the respiratory centre; hence, at the close of the operation, it is so damaged that it fails to respond to the normal stimulus. Under such conditions it may be many hours before the respiratory centre is once again able to exert control and, in the meantime, the severe accompanying acidosis—if not remedied

—will bring about circulatory failure and death. Unfortunately, at the present time there is no really simple and accurate method of measuring either the alveolar carbon-dioxide concentration or the pH of the blood that can be used routinely by the anaesthetist during anaesthesia. Nevertheless, the realization of the importance of the level of carbon dioxide in the blood in relation to activity of the respiratory centre has significantly reduced the incidence of prolonged apnoea.

Recently, a number of cases have been reported in which clinically it would seem that the neuromuscular block produced by D-tubocurarine was not reversed by an anticholinesterase drug. Typically the patient is elderly, suffering from acute intestinal obstruction, and is severely dehydrated. After operation he either fails to breathe or makes weak respiratory efforts with a pronounced tracheal tug, and later dies of circulatory failure. The term " neostigmine-resistant curarization " (Hunter, 1956) has been applied to these cases, but in the present state of our knowledge this is not justified. The reasons are that, in this type of case, the patient behaves in a similar manner when the relaxants are not used, and no evidence has been presented to prove that neostigmine does in fact fail to reverse any curarization that is present. Attempts have been made to link this type of case with a low level of serum potassium, and thus with a low intracellular potassium. It has been suggested that, in the presence of a low intracellular potassium, D-tubocurarine might pass into the nerve cells in the brain and directly depress their activity (Foster, 1956). There are, therefore, two main theories that have been propounded to explain this type of case—namely neostigmine-resistant curarization and a central action of D-tubocurarine in the presence of a low serum potassium. At the present time there is insufficient evidence available in man to support either theory.

b. Peripheral Causes

Probably the simplest, but yet one of the commonest, peripheral causes of a prolonged reaction to a particular muscle relaxant is an overdose of the drug. In skilled hands this rarely happens.

It has been shown that variations in blood flow can seriously affect the duration of action of a muscle relaxant (Churchill-Davidson & Richardson, 1952). Thus, a cooled limb with a poor peripheral circulation may stay paralysed for a number of hours, whereas a warm limb with a fast circulation is affected for only a few minutes. As the diaphragm is richly supplied with blood vessels, such a cause is not highly probable, but in cases with prolonged hypotension and a low cardiac output this possibility should be borne in mind.

The short duration of action of succinyldicholine is due to its rapid destruction by plasma cholinesterase: it has now been clearly established that a prolonged response may follow in the presence of a low serum cholinesterase level (Evans, Gray, Lehmann & Silk, 1952; Bourne, Collier & Somers, 1952). Such cases are rarely encountered, but a reaction should be foreseen in the presence of wide-spread damage to the liver, as this is the site of plasma cholinesterase formation.

Succinyldicholine is broken down in vivo into succinylmonocholine and choline. As the monocholine has about one-eightieth of the activity of the dicholine, and the hydrolysis rate is 6–8 times slower, it is theoretically possible that the monocholine derivative is capable of producing muscular paralysis in its own right (Foldes, McNall & Birch, 1954). However, it is unlikely to be able to act in this manner unless large doses of succinyldicholine—in the region of 1 or 2 g.—are used.

Lastly, there is increasing evidence that a dual block may occur in normal subjects after the administration of a depolarizing drug. It is most likely to occur following successive doses of the drug, as previously discussed. If the presence of such a block is suspected it is advisable to give an intravenous injection of an ultra-short-acting anticholinesterase drug, e.g. 10 mg. Tensilon. If the neuromuscular block increases or shows no improvement, then a depolarization block is present. If, on the other hand, there is marked improvement in muscle activity, then a dual block is present. As the action of Tensilon lasts only a few minutes, a diagnosis can easily be made without interfering with the ultimate treatment of the patient.

Despite all the causes to account for a prolonged action discussed above, it must be emphasized that there is no evidence to suggest that any of the relaxant drugs directly interferes with the metabolism or the circulation of the individual. Consequently, provided that adequate steps are taken to ensure good ventilation of the lungs, the delay in onset of respiration should be regarded as only temporary, but it is essential that biochemical analysis should be carried out during this period to ensure that normal equilibrium is being maintained.

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ANAESTHESIA FOR OBSTETRICS

Recent Advances

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1 Effect on the child
  a Thiopentone
  b Relaxants
  c Cyclopropane
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2 The danger of aspiration
  a Preventive measures
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The last twenty years have seen greater advances in anaesthesia than in most branches of medicine. Intravenous anaesthetic agents, relaxants, closed-circuit techniques and " balanced anaesthesia " have provided a considerable increase in the safety and comfort of patients undergoing routine and emergency surgery.

In centres undertaking specialized work, the more refined techniques of hypotension and hypothermia represent like advances. Yet throughout this period of rapid progress, drugs and methods routinely used in obstetric anaesthesia in the greater proportion of British hospitals have remained unchanged from those in vogue at the beginning of the century. The standard trio of drugs has been ether, nitrous oxide and chloroform; the methods of administration have been either by the open mask or by the semi-closed circuit. The effect of this situation in terms of maternal and foetal mortality and morbidity will be discussed later; in terms of maternal comfort, it can promote no self-satisfaction amongst anaesthetists. The pride which every anaesthetist takes in providing his patient with a pleasant induction, and as great a freedom as possible from post-operative vomiting and other distress, seems strangely to desert him when he is confronted with a maternity case.

This lack of progress is due to a combination of ignorance and fear nurtured by the unfortunate circumstance that anaesthetics in obstetric cases in hospitals, where progress must be initiated, are usually given by trainees. Ignorance of the ways in which modern anaesthetic agents affect the foetus made obstetricians wary of permitting their junior anaesthetist colleagues to use the newer techniques. The more responsible anaesthetists, increasingly out of touch with obstetric work as they advanced in seniority, failed to stimulate the necessary investigations.

This negative attitude had to change eventually, and the literature of the last few years reveals a mounting tide of articles suggesting methods of anaesthesia in obstetrics, involving increasing incorporation of the most modern agents.

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The main problems concern the long-held fear of neonatal depression, and the more recently emphasized danger of maternal asphyxia from aspiration. Behind both looms the general problem of lack of trained personnel—causing some concern in the smaller hospitals and in domiciliary practice in Britain, but of much greater moment in larger and relatively undeveloped countries.

1. Effect on the Child

a. Thiopentone

It has long been accepted that anaesthetic gases cross the placenta. It was held, however, that, so long as the mother was only lightly anaesthetized at the time of delivery, the child would also be only mildly depressed by ether or chloroform. Thus a general agreement existed that fluctuations in the level of depression produced by anaesthetic gases in the mother were mirrored by like changes in the foetus. Whilst, so far as I am aware, this theory was never subjected to analytical investigation, it seems probable that it is roughly correct. Thus ether and chloroform were " safe " anaesthetics—as far as the child was concerned—so long as the administrator took care to lighten anaesthesia just before delivery. The main concern was with those difficult operative deliveries which required a prolonged anaesthetic. In such cases there appeared to be a build-up of total anaesthetic in the mother—and hence in the child—which proved difficult to disperse at the critical moment, and resulted in the production of a dangerously depressed infant. This class included also those operations involving considerable pain or shock—in effect, those situations in which deep ether anaesthesia was formerly the anaesthetist's only weapon.

With the advent of the intravenous anaesthetic drugs there came a strange reluctance to apply the " maternal-foetal mirror-theory " to the newer techniques. For many years the placenta was envisaged as offering a step-like barrier to these agents. Once the threshold had been passed, the foetus was said to receive a dangerous gush of depressant drug—whatever dosage had been given to the mother. An authoritative article which set the tone for this attitude (Hellman, Shettles, Manahan & Eastmann, 1944) asserted that thiopentone did not reach the foetus until 12 minutes after administration to the mother, and that delivery after that time would result in a dangerously depressed child. The insistence on the potential danger and on the need to hurry, provoked by such rules, aroused considerable distrust of these drugs' being used at all in the labour ward.

More recent work has revealed a different picture. Articles by Cohen, Paulson, Wall & Elert (1953) and by Thomas & Gibson (1953) recounted the use of thiopentone for operative delivery without complication. The former, by comparing blood-level determinations, concluded that the longer the time taken over delivery, the less likely was the drug to cause neonatal depression; and the latter that, however long the operation, there was no " demonstrable depression of the foetus ". McKechnie & Converse (1955) and Crawford (1956a), in more detailed analyses, showed that, certainly within three minutes of administration of thiopentone, the concentration of the drug in the foetal blood equalled that in the maternal blood, and that thereafter the two levels fell synchronously. It was suggested (Crawford, 1956a) that a dose of thiopentone of 250 mg. represented a safe induction from the point of view of the foetus, however long the period

before actual delivery. It must be noted, however, that all the above-quoted articles referred to the single-dose technique.

Further accounts have appeared (Bowen, 1955; Ozinsky & Harrison, 1956) in which the use of thiopentone is tacitly accepted in discussion of other aspects of the subject; and my personal observation leads me to the conclusion that fewer anaesthetists now are denying their patients this most rapid and pleasant induction on the grounds of potential danger to the foetus.

b. Relaxants

Gray (1947) first demonstrated that D-tubocurarine did not cross the placental barrier. Recently, the use of relaxant drugs has increased greatly in operative obstetrics: as an aid to intubation—a vital part of many of the methods in popular use—and as a factor in the production of balanced anaesthesia. Coleman & Day (1956), Collier (1956), Bowen (1955), Walter (1956), and Azzopardi (1956) all report the successful use of D-tubocurarine and gallamine, though it must be noted that, whilst the former has been shown not to be passed to the foetus, gallamine (Crawford, 1956a) does get through in quantities which might, under some circumstances, prove dangerous. No analysis of foetal blood for succinylcholine has been reported, but there are many testimonies to its use without effect on the child (Coleman & Day, 1956 ; Davies, 1955; Molphy, 1955; McNab, 1955).

c. Cyclopropane

Cyclopropane, like thiopentone, failed for many years to become popular as an anaesthetic agent in obstetrics. There are now, however, many accounts of its use (Collier, 1956; Bowen, 1955; McNab, 1955; Coleman & Day, 1956; Crawford, 1956a, 1956b) both for induction and—after thiopentone induction—for maintenance of anaesthesia. The rapidity of excretion of cyclopropane, and the ease with which a light plane of anaesthesia may be maintained, make the occurrence of neonatal depression much less likely than when ether is used. Its main drawback is that it cannot be employed in domiciliary practice without the use of cumbersome or expensive apparatus. In addition, it has been demonstrated that there is an increased tendency for cardiac irregularities—and cardiac arrest—to occur in patients who are given cyclopropane shortly after the administration of pitocin. As pitocin therapy is becoming rapidly more popular, anaesthetists generally should be aware of this danger, and should be prepared to substitute nitrous oxide for cyclopropane in cases in which inquiries prove pitocin to have been given.

d. Pethidine and Chlorpromazine

These drugs in combination present the latest and one of the most attractive possibilities in anaesthesia for obstetrics. It is now generally agreed in Britain that, by following roughly the suggestions made by Roberts (1948) in her original article on the subject, a quantity of pethidine may be administered which is sufficient to provide adequate analgesia for the mother, but which is unlikely to cause undue neonatal depression. A general rule has been formulated that the drug should be avoided if delivery is expected within two or three hours. However, with the introduction of the antagonists of morphine, there has arisen a tendency to waive this rule, on the grounds that a drug such as levallorphan may readily be given should the infant appear to be depressed by pethidine. It is to be noted that, in one of the latest reviews of the effects of analgesic drugs used in child-birth (Roberts, Kane, Percival, Snow & Please, 1957), pethidine appeared to exert less depressant action on the respiratory activity of the child when given within three-and-one-quarter hours of delivery than when the time-lag was greater.

Chlorpromazine is rapidly achieving popularity for use during labour (*Lancet*, 1956; Hershenson, Koons & Reid, 1956; Anz & Smith, 1956; Schaffer, 1956; Norton, Weingarten & McDonough, 1956; Karp, Lamb & Bonaron, 1955; Savage, 1955; Stewart, 1956; Browne & Mannion, 1955; O'Keefe, Shanahan & Quane, 1955). Its general uses in the scheme of analgesia are not for discussion here, but it has been strongly claimed (Davies, 1955; Carroll & Hudson, 1955) that no ill effects on the foetus are to be observed. Davies (1955), in his reported series of 100 cases, gave chlorpromazine in doses of 25 mg. at an interval which averaged 74 minutes before delivery of primiparae, and 44 minutes in the multiparous patients. He further refers to five subjects of Caesarean section who each received 25 mg. of chlorpromazine intravenously 30–45 minutes pre-operatively, anaesthesia being induced and maintained with a nitrous oxide–oxygen mixture, plus succinylcholine when relaxation was required. Scott & Gadd (1957), in a discussion on the use of Kielland's forceps under local analgesia, refer to 39 patients to whom they gave, in addition to local analgesia, 100 mg. of pethidine and 25 mg. of chlorpromazine intravenously. They observed no ill effects from this, and suggest that it is probable that a considerable number of forceps operations could be performed under this drug alone.

Work in progress (Crawford, unpublished) seems to indicate that a mixture of pethidine (150 mg.) and chlorpromazine (50 mg.) given intravenously in 15–20 ml. of solution 10–15 minutes before operative delivery is commenced, may be considered safe from the point of view of the child. The merits of the method with regard to the mother will be examined in the succeeding section.

It does indeed seem that the keynote of recent advances in obstetric anaesthesia is that struck by Hellman & Hingson (1953). They concluded that, whilst it is true there is a significant difference in the reaction of infants, as determined by the breathing and crying times, according to whether or not the anaesthetic used crosses the placenta in significant amounts, yet whether or not the anaesthetic crosses the placenta does not appear to influence the perinatal infant loss, either for normal-term infants or those jeopardized by prematurity or Caesarean section. This conclusion, of course, carries the proviso that the drugs are used in safe quantities, and are administered by trained workers.

2. The Danger of Aspiration

With the advancing standards of obstetric care and understanding there has been a steady decline in maternal mortality in Britain. This has served to draw increasing attention to the number of deaths due to anaesthesia. It is said (Parker, 1956) that, in 1952, 4% of maternal deaths in England and Wales were related to anaesthesia. Klein, Clarr, Tamis & Solkow (1953) attributed 9% of maternal deaths in the Bronx, New York, during 1946–51, to anaesthesia. Although the direct causes cover the same range as in other branches of surgery, death from the aspiration of vomit or regurgitated material figures much more prominently in operative obstetrics. In Klein's series 7 out of a total of 9 deaths associated with general anaesthesia were due to this cause, whilst Wylie (1955)

noted that, in a series of 31 maternal deaths from anaesthesia, 15 were due to aspiration asphyxia.

It has long been held that there is a delay in the gastric emptying time of the woman in labour. This, combined with the tendency of the relatives to encourage the taking of food by these patients and the fact that most obstetric operations are emergencies, is considered an important factor in producing a high vomiting rate. An attempt has recently been made to show, however, (Crawford, 1956b) that, in fact, there is the same variation in the rate of gastric emptying in women in labour as in normal subjects, and that if, during the first stage of labour, a semi-fluid diet is given, there need be little fear of an increased danger of vomiting or regurgitation.

However, this does not influence the acid-aspiration syndrome (Mendelson, 1946), which essentially is a rapidly developing bronchopneumonia due to the aspiration of a small quantity of regurgitated, highly acid gastric contents. This implies, of course, the reverse of the " delayed emptying " theory, for now the mother's stomach has somehow remained empty during labour except for its own acid secretion. Despite their divergences, these suggestions are often loaded into the same shot during debate. It is interesting to note that, recently, yet a third theory has been advanced by Hausmann & Lunt (1955). They suggest that the acid-aspiration syndrome is actually due to acute adrenal deficiency, following the sudden loss of adrenocorticotrophic hormone and gluco-corticoids produced by the placenta. Surely, though, these patients die, not because they present a peculiar anaesthetic problem, but because they have been badly anaesthetized.

a. Preventive Measures

Many ways of combating the possibility of aspiration of vomit have been advocated. There is a school which holds that all patients for Caesarean section—and possibly for forceps delivery too—should be intubated (Wylie, 1955; Collier, 1956; Coleman & Day, 1956). Yet there must always be a delay between giving the relaxant and inflating the cuff, and under the circumstances postulated by these workers this might well be dangerous. Furthermore, many of this school will agree that much of the trouble arises from the occasional administration of the anaesthetic in obstetric cases by inexperienced anaesthetists—so there is little point in advocating, for such personnel, a method requiring even more skill.

Many anaesthetists urge that, on the analogy of the pre-operative treatment of acute intestinal obstruction, the patient's stomach be emptied via a large tube before the anaesthetic is commenced. This is probably sound policy if the patient has not had the benefit of intelligent supervision of her diet during labour. It is undoubtedly uncomfortable, but so are the sequelae of inhaled lumps of food. Its effects on the occurrence of the acid-aspiration syndrome are unrecorded. As an alternative, Holmes (1956) suggests giving apomorphine to effect a less unpleasant emptying of the stomach.

b. Local Analgesia

Undoubtedly the dangers of aspiration asphyxia are, other than in exceptional cases, avoided by the use of local analgesia. Spinal (Adatia, 1955), extra-dural (Dawkins, 1956) and caudal (Fitzgerald, 1956) analgesia all have their advocates for Caesarean section and forceps delivery; whilst pudendal block is strongly recommended for forceps delivery by Hibbard & Grassie (1955), Scott & Gadd (1957) and Malkani & Sikand (1956), amongst others. These methods, however, do have their limitations.

It is generally agreed that pudendal block is unsatisfactory for patients requiring appreciable internal manipulations, though Scott & Gadd (1957) claim that their method—using Kielland's forceps—is satisfactory. The spinal and extra-dural methods are specialized techniques carrying inherent risks of grave complications, and, unlike pudendal block, are not readily applicable to domiciliary practice. Despite these objections, a strong case could be made for the use of a conduction block in districts suffering from a shortage of trained doctors.

c. General Anaesthesia

The choice of drugs for producing general anaesthesia must be governed by common sense and not, as tends to be the case, by a rather partisan-like fervour, usually displaying a strict conservatism. The best agent in given circumstances is that with which the anaesthetist concerned is most familiar. Apart from this, which should be the governing factor, the main debate centres upon the merits and demerits of ether and thiopentone. Ether is well recognized as the anaesthetic in general use most likely to provoke vomiting. Especially is this so during the stormy induction phase for which, in unskilled hands, it is notorious, and when the patient is only lightly anaesthetized (as will be the case at the time of delivery). Today, few anaesthetists obtain, by choice, much experience in producing smooth ether anaesthesia. For these reasons it has been suggested (Crawford, 1956b) that ether is an important cause of the morbidity and mortality under discussion.

Members of the junior resident staff in British hospitals seem to be encouraged to practise ether anaesthesia in the labour ward (with or without a nitrous-oxide introduction) whilst they are learning the more modern techniques in the general operating theatre. This must be an important reason for Parker's finding (*Lancet*, 1955) that in Birmingham, during a 10-year period, the risk of maternal anaesthetic death was greater in hospital than in domiciliary practice. There seems to be no good reason why patients should be denied—considering both comfort and safety—an induction with thiopentone or, if an inhalational anaesthetic must be used, cyclopropane. If the regime of a rapidly digested diet has been in force, such a method will avoid vomiting and its attendant dangers—an incidental point is that an artificial airway should not be used, as pharyngeal stimulation is likely to provoke vomiting during the period of lightened anaesthesia, whether the maintenance is with nitrous oxide or cyclopropane. If a heavy meal has been given during the four to six hours before operation, then, as in any emergency case, the stomach must be emptied before a general anaesthetic is given. Intubation, with its critical delay period and its demand for skill, has little to commend it in this field.

d. Chlorpromazine and Pethidine

In this most recent method for forceps delivery, a mixture of pethidine (150 mg.) and chlorpromazine (50 mg.) is given by slow intravenous injection. The patient is allowed to settle down for 10–15 minutes, and then placed in the lithotomy position. It has been found helpful to infiltrate the line of the episiotomy with about 10 ml. of lignocaine (1%) before starting the operation. Although about 20% of patients appear to offer some protest during delivery, in all the cases so far encountered there has been amnesia for the whole event. The patients are rather drowsy for two or three hours after

operation, but may be engaged in conversation. No alarming changes in blood pressure have been recorded and, of course, nausea and vomiting have been markedly absent. At the time of writing it is felt that the more difficult internal manipulations might not be possible under this " hypo-aesthesia ", but our views might change. Highly nervous or excitable patients have not proved particularly amenable to the method, and it has not been used for Caesarean section—though probably it would be possible to use it if combined with local skin infiltration. With these provisoes, the pethidine and chlorpromazine combination appears to offer the possibility of a considerable advance in anaesthesia for obstetrics, especially for domiciliary practice or in small country districts.

3. Conclusion

In summary, it is suggested that thiopentone (about 250 mg.) induction, with cyclopropane or nitrous-oxide maintenance, and with neither intubation nor airway, is the most satisfactory form of general anaesthesia from the point of view of both mother and child. Dietary care is of prime importance during labour, and indiscretions must be corrected before induction of general anaesthesia. Pudendal block is the best alternative method for forceps deliveries, but the production of a pethidine and chlorpromazine hypo-aesthesia might prove to be the answer to our main problems in anaesthesia for obstetrics.

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NEONATAL ANAESTHESIA

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1 Respiration in the new-born
2 Response to relaxants
3 Anaesthetic procedures
4 Post-operative care
5 Incidence of surgery in neonatal period
References

Any discussion on neonatal anaesthesia should start, perhaps, with a consideration of the new-born infant as a subject for anaesthesia and surgery. At birth the infant is not as yet completely adapted to an extra-uterine environment. His heart has hitherto been called upon to provide a circulation through his own tissues, and at the same time through a large additional mass of placental tissue, and the foetal circulation is so routed that the burden of this load is divided between the right and left sides of the heart. It might, therefore, be expected that at birth the heart would be a well-developed organ, well able to provide the energy required for the lesser work it is now required to do. This is well illustrated by its size. The cardio-thoracic ratio of the new-born infant is on the average 0.55, as compared with an upper limit of normality of 0.5 for the adult. On the other hand, the respiratory system at birth is primitive as compared with that of the adult.

1. Respiration in the New-Born

Engel (1947), in a histological study of the child's lung, showed that at birth the area available for respiratory exchange in the new-born infant's lung was small, both when considered as a function of volume of lung tissue, and when considered in relation to the body-weight of the organism. The oxygen consumption of the infant at rest, per unit of body-weight, is high as compared with that of the adult. It follows, therefore, that the rate of gaseous exchange per unit of respiratory surface is also very high, a factor which is influenced in the same direction both by the small respiratory surface and by the high oxygen consumption per kg. of body-weight.

It has long been known that most new-born infants will, if observed for a long enough period, exhibit a periodic type of respiratory rhythm. This type of rhythm is shown by adults in a variety of states: it is seen when the adult respires an atmosphere with a low oxygen tension, it is seen in alkalaemic states, it is seen when the respiratory centre is depressed by drugs or other causes, and it is seen in patients with certain pulmonary diseases in which there is a deficiency of gaseous diffusion. In other words, it is seen in those circumstances when the primary driving stimulus of the respiratory centre is oxygen deficiency rather than, as normal, carbon-dioxide build-up. It is also known that, in cases of diffusion deficiency, it is oxygen uptake which becomes inadequate before carbon-dioxide output fails.

It is tempting to suggest that the new-born infant exhibits this periodic breathing because his limited respiratory surface can deal more adequately with his carbon-dioxide output than with his oxygen uptake requirements, in the same way as can that of an adult respiratory system in which there is a pathological limitation of gaseous exchange. It has been shown that the administration of oxygen will abolish the periodic breathing of infants when it is present, and this would suggest the truth of the hypothesis that periodic breathing is the result of the primitive nature of the infant's lung.

In addition to possessing a lung as yet ill-adapted to its function, the infant has a type of ventilation which is more primitive than that of the adult. Observation of the thoracic radiograph of any infant will show that the ribs tend to have a much more horizontal disposition than those of the adult. The significance of this fact is that rib movements can contribute less to changes in thoracic volume than can the more oblique adult ribs, and respiration is, of necessity, largely diaphragmatic. This type of respiratory activity is responsible for collapses of the upper lobe so frequently seen post-operatively in the new-born, and discussed in section 4.

In spite of his almost entire dependence on his diaphragm for respiratory exchange, the new-born infant is capable of producing, by his respiratory efforts, quite high negative intra-thoracic pressures. There is an interesting physical explanation for this which is rarely appreciated. It is that the negative intra-thoracic pressure depends ultimately on the tension of the spherical surfaces of the diaphragm. It can be mathematically shown that the pressure difference between the two sides of a spherical membrane in tension is, for any given degree of tension, inversely proportional to the radius of the sphere. Thus, for any given degree of tension of the diaphragm, the infant produces higher negative intra-thoracic pressures than the adult, because, by virtue of his small size, the radii of his diaphragmatic curves are smaller.

The negative intra-pulmonary pressure which can be achieved is 40–50 cm. H_2O, and it would seem that, this being so, there is little danger in applying intermittent positive-pressure ventilation to even the smallest infants. This statement should, perhaps, be qualified by saying that occasionally the premature infant produces alveolar rupture and interstitial emphysema by his own respiratory effort, and presumably the same effect could result from intermittent positive-pressure ventilation, even at physiological pressures; but it is doubtful that this would occur if the lung were normal.

The relative immaturity of the respiratory system in general, as regards both lung structure and ventilation mechanics, has been stressed in this paper in order to emphasize the necessity for maintaining ventilation at a high rate during anaesthesia and to illustrate the desirability in the new-born of artificial ventilation. Provided that this ventilation is maintained, without extra effort on the part of the child, then the circulation will remain intact if the blood loss is made good. It must be appreciated that the circulatory system of the new-born is adapted to function without any pressure differential between the intra-thoracic and extra-thoracic circulations and, this being so, the arguments against intermittent positive-pressure ventilation on the grounds of interference with circulatory dynamics lose much of their weight.

If the desirability of controlled ventilation of the new-born

anaesthetized child is accepted, it is necessary to consider the methods whereby this is achieved.

2. Response to Relaxants

It has been pointed out in the past that apnoea can be readily produced in the new-born by intermittent positive pressure in the absence of deep anaesthesia and relaxant drugs, and it has been stated that, this being so, there is little indication in this age-group for these latter drugs. As this statement was made at a time when the only satisfactory relaxants were of the anti-depolarizing type it was substantially true then. However, in more recent times the introduction of the newer relaxants has changed the picture somewhat.

A study of the response of the new-born to the relaxant drugs has been made by Stead (1955). The findings showed that the new-born infant exhibited a response to an anti-depolarizing drug which was greater than that shown by the adult to a comparable dose of the drug, that is, a dose of 0.078 mg./kg. of D-tubocurarine chloride produced in the new-born a complete and prolonged apnoea; yet in the adult this dose normally produces no objective effect and minimal subjective effects. On the other hand, a depolarizing drug, succinylcholine chloride, when given in a dose which, weight for weight, will produce several minutes of apnoea in the adult, produces only a transient depression of respiration in the new-born. The finding of this sensitivity to D-tubocurarine chloride explained certain reports (Roberts, 1950; Rickham, 1952) of infants receiving D-tubocurarine chloride in the course of anaesthesia and failing to regain adequate respiratory function. In view of this relative hypersensitivity to D-tubocurarine chloride it has been suggested that drugs of this type should not be given to the new-born, and that, should relaxants be given, they must be of the depolarizing type.

It must be appreciated, however, that one cannot strictly speak of a depolarizing drug, because a drug which might initially produce a neuromuscular block by a depolarizing effect may subsequently produce what has been termed the " dual response "; that is to say, the nature of the myoneural block changes from a depolarization type to an anti-depolarization type. One might expect that an organism which is sensitive to anti-depolarizing agents might be particularly prone to exhibit the dual response with depolarizing drugs. This appears to be so in the case of the neonate, where there may frequently be seen a depressed respiration following succinylcholine administration over a long period—a depression which may be antagonized by either neostigmine or edrophonium chloride; this shows the depression to be due to an anti-depolarizing effect.

This depression appears to be related to the total dose of the drug, and this has a bearing on its mode of administration. It is impracticable to administer relaxant drugs to small infants by continuous intravenous infusion because concentrations of the drug in the infusion low enough to give accurate control of the dosage demand the infusion of dangerously large volumes of fluid. One is, therefore, forced to adopt intermittent injection techniques. If the blood level of a drug, such as succinylcholine, is to be maintained over a period of time above a certain minimal value, the total dosage required increases enormously if the drug is given intermittently rather than continuously, by virtue of its enzymic destruction in an exponential fashion. In the case of succinylcholine large doses greatly predispose to the development of a dual response; therefore an attempt should be made to approach the state of affairs which would exist with continuous administration by giving small and frequent doses rather than larger and less frequent ones. The answer to these practical difficulties might well be the use of a mechanically controlled slow-injection syringe.

The nature of the new-born infant's sensitivity to anti-depolarizing effects is obscure, but there is some recent work on the metabolism of neonates which might have a bearing on this subject. Rickham (1957), making observations on the metabolic response to surgery in the new-born, observed that in this age-group there is, as a response to trauma, a loss of potassium and of nitrogen from the body, as is seen in adults (Moore & Ball, 1952), but in the case of the adult the ratio of potassium loss to nitrogen loss (K : N ratio) is greater than the normal ratio in which these elements exist in normal tissue. This indicates a breakdown in tissue and, in addition, there is a loss of potassium from tissue which retains its nitrogenous content. The K : N ratio in the new-born, as determined by Rickham, is approximate to that of lean muscle tissue—an indication that cellular breakdown occurs, but that there is little loss of potassium from cells which do not break down. The fact that the new-born infant is sensitive to anti-depolarization effects, which depress the ionic migration at the end-plate, and is resistant to depolarizing effects, which result from ionic migration, may well be related to the infant's resistance to intra-cellular potassium loss in response to the hormonal effects of trauma.

There is another interesting finding in relation to the relative sensitivity to the different types of relaxant drug, in the work of Wilson on myasthenia gravis. Wilson (1955) was able to extract from the thymus glands of patients with myasthenia gravis, who exhibited responses to relaxant drugs similar to those of the new-born, a substance which in vitro would sensitize mammalian muscle to the effect of D-tubocurarine chloride. He was able to extract from foetal thymus glands an apparently identical substance. This might be thought to be the reason for the similarity in the response of the neonate and the myasthenic to the relaxant drugs.

3. Anaesthetic Procedures

If the advantages of controlled respiration are to be exploited in anaesthesia for the new-born it is necessary, of course, for endotracheal intubation to be carried out. This may present certain technical difficulties in the smallest infants, and it is, therefore, unwise that it should be performed under the effect of relaxant drugs. This might appear to be irrational, for the technical difficulties would obviously be reduced by these drugs, but even with their help the procedure might be prolonged and one might well be left with an apnoeic infant whom one could not readily ventilate. The induction of anaesthesia by inhalation methods to sufficient depth to eliminate laryngeal spasm during the manipulations may be very difficult and prolonged in infants. It is, therefore, recommended by many authorities that the intubation should be performed before the induction of anaesthesia. In this way the hazards of regurgitation and aspiration are minimized, and anaesthesia can be quickly induced. Following intubation, the child may be saturated with nitrous oxide as rapidly as possible by intermittent positive pressure, and the relaxant drug may then be administered. In this way perfect operating conditions are obtainable, and the more potent and, therefore,

more toxic agents are eliminated from the anaesthetic technique. In order to carry out such a procedure it is obviously necessary to have ready access to the venous system and this can be obtained only with an intravenous infusion. To eliminate the necessity for flushing-in drugs introduced with the infusion tubing it is desirable to introduce some form of three-way tap near the cannula to ensure positive injection.

The conditions for which surgery in the early days of life is required may be classified into three main groups from the point of view of the anaesthetist:

i. Operations for the relief of respiratory obstruction.
ii. Operations for the relief of alimentary obstruction.
iii. Operations for the relief of miscellaneous conditions, i.e., anomalies of the urogenital tract, exomphalos, meningomyeloceles, and so forth.

It is the first group that is of prime importance to the anaesthetist in that the condition may call for emergency anaesthetic treatment on admission. These conditions will, therefore, be discussed in greater detail.

Post-Choanal Atresia

Non-patency of the posterior nares, or post-choanal atresia, can be a source of such severe respiratory obstruction in the new-born as to endanger life. Clearly, the emergency management of these cases is the establishment of an oral airway, which may be done with an oropharyngeal airway or an endotracheal tube, which may be left in situ until the condition is treated surgically.

Micrognathia

In this condition there is such gross underdevelopment of the lower jaw that the tongue is forced back on to the posterior pharyngeal wall in severe cases, and produces obstruction to respiration. This may be relieved by nursing the child in the face-down position with head extension, or may require emergency endotracheal intubation.

Subglottic Stenosis

This is one of the respiratory obstructions of interest to the anaesthetist as it is often discovered for the first time when an endotracheal tube is introduced for resuscitative measures. I have met two such cases. In both, the endotracheal tube was passed through the vocal chords, but would not pass down the trachea. In one of these cases, at a subsequent post-mortem examination, the trachea would admit only a fine probe.

Respiratory Obstruction due to Vascular Ring

Congenital anomalies of the aorta, in which there is a persistence of both the right and the left primitive arches, may result in respiratory obstruction at birth, and require emergency treatment by the anaesthetist.

Diaphragmatic Hernia

Congenital diaphragmatic hernia can give rise to respiratory obstruction shortly after birth in that the bowel is within the thoracic cavity and, on becoming filled with air, as it does after birth, may by occupying space in the thorax give rise to respiratory embarrassment. In many of these cases, intubation and assisted respiration are required until the condition can be dealt with surgically.

The other conditions for which surgery is performed in early life have no special interest for the anaesthetist, with the exception of oesophageal atresia with tracheo-oesophageal fistula. By far the most common type of this anomaly is the one in which the upper end of the oesophagus ends blindly and the lower end communicates through a fistula with the trachea. Infants with this condition can become very distended because, by their respiratory efforts, they pump air from the trachea to the gastrointestinal tract. Some cases are reported where there has been a paradoxical type of respiration between stomach and lung. Intermittent positive-pressure ventilation can, in such cases, result in gastric distension, but this is relieved at an early stage in the operation and is a hazard of lesser magnitude than intra-thoracic surgery without controlled ventilation. I feel that the possibility of the occurrence should not tempt us to perform this operation under an anaesthetic technique in which respiration is spontaneous.

During the course of surgical procedures, infants tend to become hypothermic; in most cases this is not a serious occurrence and it could be argued that it is desirable. There is, however, the condition of sclerema to which hypothermia, especially in premature infants, may give rise. Sclerema was recently reported to have developed in infants who had been exposed to cold, and admitted to hospital in a hypothermic state. It is certainly true to say that sclerema is often a fatal complication of surgery in premature infants and, therefore, it is considered wise to modify the fall in temperature during the course of operation by the supply of external heat. My practice is to place an electric pad under the infant.

4. Post-Operative Care

Post-operatively, infants should be nursed in an atmosphere of high humidity or at a controlled temperature of about 85° F.[1] The high humidity has the advantage of reducing pulmonary complications, and the additional advantage of reducing the insensible fluid loss and thus making the maintenance of fluid balance easier. It has been estimated that the insensible fluid loss of the infant in an atmosphere of 100% humidity is about 14 ml. per day (O'Brien, Hansen & Smith, 1954).

Normally the infant loses as much as 10% of his body-weight in the first days of life, and losses of as much as 20% do not appear harmful (Smith, 1951). Thus when nursed in an incubator at high humidity, neonates who are not initially dehydrated can be left for 2–3 days post-operatively without fluid intake. Gross & Ferguson (1952) have recommended infusion at the rate of 30 ml. per lb.[2] body-weight per day during the post-operative period, but Rickham (1957) suggests that even this is too high and that 5 ml. per hour (120 ml. per day) is adequate for the fluid requirement on the first two post-operative days, and thereafter 10 ml. per hour (240 ml. per day). These figures are for full-term infants, and are reduced for premature infants.

The new-born infant has great difficulty in excreting salt, so that the infusion solution should never be normal saline. Rickham suggests ¼ normal saline as the basic solution. By virtue of the infant's different metabolic response to surgery (see above), his potassium loss and, therefore, requirements, post-operatively are minimal and none need be given in the absence of intestinal suction; if gastric suction has been continued for three days, 60 ml. of Darrow's solution per day are quite adequate for the requirements of full-term infants.

[1] t° F. = ⅝(t − 32)° C.—Ed.
[2] 1 lb. = 453.59 g.—Ed.

The avoidance, if possible, and prompt treatment when not, of post-operative pulmonary complications is vitally important if a low rate of mortality is to be achieved in the field of neonatal surgery. It has been mentioned earlier that the construction of the neonate's thoracic cage is such that the ventilation of his apical lobes is less efficient than in the adult. The appreciation of this fact is of some importance in the post-operative management of infants. When an infant gets a post-operative pulmonary collapse it is invariably the upper lobe which is affected first. The collapsed area may later extend to a lower region, but the primary centre of the collapse is always the upper lobe. It is, therefore, important to take every precaution possible to ensure that such a collapse does not occur. The high humidity is one helpful factor, but posture is perhaps more important. The infant should be nursed with a head-up tilt, that is to say, not with the head and shoulders propped on pillows, but with the infant lying on a flat tray, the whole of which is tilted. In addition the child should be nursed on the side (because otherwise the head-up tilt would be hazardous as regards the risk of aspiration), and should be turned from side to side at three-hourly intervals.

Should a pulmonary collapse occur in spite of these measures, then the child should be postured with the collapsed lung uppermost, and the chest lightly tapped with the fingertips for about three minutes each hour. If the lung does not re-expand after about six hours of this regime, it will be necessary to aspirate the tracheobronchial tree. This may be done by passing a fine urethral catheter (No. 3 English gauge; diameter 2.5 mm.) down the trachea, and if the right lung is affected this will probably clear it. If, however, the collapse is on the left side it is probably desirable to perform bronchoscopic aspiration. This is readily performed if a bronchoscope of 3.8 mm. diameter and about 18 cm. long is available. The good results of aspiration are usually dramatic.

Much has been written of the hazards of using oxygen in infants, and particularly in premature infants, as an aetiological factor in the development of retrolental fibroplasia. Despite this, I believe that in the first twenty-four hours after operation there is little risk of damage occurring, as the respiratory movement is usually limited and, therefore, oxygen is introduced into the incubator atmosphere for this time, and longer in the presence of pulmonary complications. In some 500 cases treated in this way there has been no case of retrolental fibroplasia.

5. Incidence of Surgery in Neonatal Period

Working in a unit which serves an area with a population of a little over two million, my associates and I anaesthetize a little over 100 neonates for major surgery each year. Translated into terms of the total population of the United Kingdom, one would expect that the number of children requiring surgery in the country as a whole would approximate to 2,000. It will thus be appreciated that the problems of neonatal anaesthesia are by no means rarely encountered. Furthermore, the relative importance of this branch of anaesthesia is increasing, for it has been pointed out (Rickham, 1952) that, as the infantile mortality becomes less, as it has done consistently in the last 40 years, the congenital anomalies are the cause of a more significant proportion of the total deaths. As the Ministry of Health Report on neonatal mortality (1949) states, "... these subjects offer great scope for research. Many can play a part in this work—paediatricians, obstetricians, anaesthetists, physiologists, social workers—indeed all those who are interested in the life and health of the next generation."

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ANAESTHESIA IN BURNS

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Burns may be classified as those with: (i) partial skin loss and (ii) whole skin loss. The former will heal without operative treatment and need not be further considered here. Small burns which destroy the whole skin may need grafting but no special features are likely to arise in considering anaesthesia for such operations.

The surgical treatment of large burned areas does, however, give rise to a number of unusual anaesthetic problems. In the early stages, oligaemic shock will occur unless the large volume of continuing plasma loss is replaced or brought to a halt. Destruction of red cells will lead to anaemia unless correction is made by transfusion of whole blood. Pain and the psychological trauma caused by the terrifying experience tend to cause restlessness and anxiety, a condition that may have been treated by one or more subcutaneous injections of morphia; this treatment will not become effective until the peripheral circulation has improved, when sudden absorption may cause collapse of such a degree as to demand the administration of a specific antagonist, *n*-allylnormorphine. The oft-repeated admonition to give morphia in small doses only, and intravenously, in shock cannot be overstressed.

Anoxia may be due to glottic oedema secondary to burns of the upper air passages or the face and neck; it may be due to oedema or atelectasis of the lungs resulting from the inhalation of steam or smoke (Finland, 1951); or it may be due to the inhalation of carbon monoxide, a condition seen fairly commonly in the burns of colliery explosions. Restlessness and anxiety arising from anoxia must be treated by efficient oxygen therapy (Shannon, 1955).

It is clear, then, that anaesthesia in this early stage has special difficulties, but it is very much in the patient's interest that dead tissue be replaced by grafts as soon as the diagnosis of destruction involving the whole thickness of the skin has been confirmed.

Burns of certain sites such as the face, eyes, hands and feet will require to be treated in the several ways outlined by the subcommittee of the British Association of Plastic Surgeons (*Lancet*, 1954). These will usually demand delayed surgery, with the exception that burns of the eyes and eyelids may require urgent tarsorrhaphy or outlay grafting.

The age and weight of the patient, and the percentage of the surface area burned, are figures upon which are based the prognosis (Bull & Fisher, 1954) and the accepted formulae for the administration of fluids (Cope & Moore, 1947; Wallace, 1951; Evans, Purnell, Robinett, Batchelor & Martin, 1952). The calculation of percentage is simplified by the use of a chart for the recording of burn distribution (Lund & Browder, 1944) and the anaesthetist will orientate himself to the problem he faces by its use. In the absence of a chart, Wallace's " Rules of Nine " (Wallace, 1951) is adequate.

1. Primary Excisions

Prendiville (1954) advocated the treatment by excision of localized full-thickness burns as soon as preparations were completed for surgery, and of extensive (over 20%) deep burns on the fifth or sixth day. A trial series of primary excisions has been carried out on large burns in the Birmingham Accident Hospital, at the earliest practicable time after the injury. This has usually been eight hours from admission, during which time: (i) a full inspection is made to estimate the extent of the burn and the proportion and distribution of whole skin loss; (ii) transfusion is begun, and plasma and red cell loss is compensated; and (iii) a blood volume estimation (Davies, 1956) is made to ensure complete resuscitation and to provide a base-line for transfusion during operation.

A similar technique has been adopted in Chepstow, with the exception that blood volume estimations have not been made. Where facilities for this estimation are not readily available the nomogram of Topley & Jackson (1957) provides useful information based upon the age, the body-length, and the approximate weight (Russell, 1949). It is seldom possible to weigh a severely burned patient to obtain an exact figure.

As an intravenous drip is running when the patient comes to the theatre, anaesthesia may be induced by intravenous injection even in small children. One of the various modifications of Gordh's needle (Gordh, 1945) should be used to avoid damage to the transfusion tubing and the consequent inability to apply pressure (Davies, 1952a; Køster, 1953; Mathy & Hügin, 1955; Middleton, 1957a).

a. Anaesthetic Requirements

The requirements of anaesthesia during these operations comprise: (i) a considerable degree of analgesia, because of the traumatizing effect of excision of large areas of skin and the cutting of equally large areas of skin grafts; and (ii) a relatively light narcosis, as it is essential that recovery from anaesthesia be rapid.

That these requirements have been met adequately by a number of widely differing techniques will be seen from the methods favoured by us in this paper, but undoubtedly the familiarity of the anaesthetist with the drugs he uses is of paramount importance. The broad principles of care in the choice of drugs given before operation, of regulated dosage of the anaesthetics used, and of continuous supervision of resuscitation, are nowhere the subject of disagreement.

The anaesthetic management of major burns is liable to be complicated by the need to supervise one or more transfusions,

and by the need for the estimation and replacement of blood loss. The single-handed anaesthetist will find that a technique in which spontaneous respiration persists will be advisable.

A sleep dose of thiopentone followed by nitrous oxide–oxygen with trichloroethylene and/or pethidine has been used most often. A small dose of suxamethonium has been used for intubation when required. This, and a technique involving the use of chlorpromazine, are preferred by one of us (L.J.W.). The last-named drug has the advantage of ensuring a smooth, light narcosis with a quiet, gradual recovery. If the dosage is correctly gauged, adequate narcosis and analgesia can be provided by the addition of nitrous oxide and pethidine for the maintenance of anaesthesia, with recovery of consciousness without excitement soon after discontinuance of the nitrous oxide.

The technique favoured by one of us (H.G.M.) is that of a sleep dose of thiopentone, followed by nitrous oxide and oxygen supplemented by gallamine triethiodide. For infants and those in whom veins are not available, induction is by cyclopropane and oxygen and maintenance by methyl *n*-propyl ether and oxygen (Middleton & Picken, 1954). More than 99% of such patients are intubated.

b. Blood Loss

Blood lost during operation is estimated by swab weighing and is replaced concurrently. In large primary excisions (over 20% of the body-surface) transfusions of quantities equal to or exceeding the entire blood volume are commonly required. Subsequent blood volume estimations confirm the accuracy of replacement.

Most major burns involve limbs. A considerable saving of blood may be effected by the judicious use of tourniquets. If necessary, sterilized Esmarch's bandages may be applied by the surgeon or a pneumatic cuff may be inflated after elevation of the limb.

The release of such a tourniquet after a period of application may cause a considerable hyperaemia in the limb with a resultant loss of blood from the general circulation and the occurrence of sudden and severe shock. More than one tourniquet should never be removed at the same time.

The effects of reactionary hyperaemia may be diminished by elevation of the limb and by the application of packs and firm pressure. If the operation permits, a crêpe bandage may be applied from the periphery in a proximal direction.

c. Induced Hypotension

Hypotension may be induced with advantage in suitable cases. Many burns occur in the very young in whom hypotension is technically difficult and a larger number occurs in the elderly (Colebrook, Colebrook, Bull & Jackson, 1956) in whom the induction of hypotension is contra-indicated.

A small series of twenty-six patients ranging in age from fifteen weeks to fifty-nine years has been anaesthetized by one of us (H.G.M.) using elective hypotension induced by trimetaphan, the largest excision being of 26% full thickness. The clinical impression gained in these procedures has been very favourable and an economy of as much as 60% of the expected blood loss has been achieved (Middleton & Kunz, 1956). One death occurred thirty hours after full recovery from anaesthesia in a patient suffering from extensive burns. Necropsy showed no findings relevant to the technique.

Elective hypotension appears to facilitate the natural process of clotting and to provide a good base for the reception of skin grafts.

2. Delayed Surgery

Despite the formidable nature of the procedures in primary excisions, anaesthesia has proved uneventful so far and the post-operative condition has been very good. The general condition of these patients before operation when they have been adequately transfused is very satisfactory. Not nearly so favourable is the picture of the patient presented for operation, as is more usual, two or three weeks after injury when definition of the burn has occurred.

A relationship has been shown (Tempest, 1956) between overcrowding and an increase in the incidence of burns and scalds in children, and of the association of illness—chiefly epilepsy and cardiovascular accidents—with burning. Middleton (1957b) found that 10% of 500 burned children were convalescing from, suffering from, or incubating infectious diseases. The seasonal increase in domestic burns parallels the prevalence of upper respiratory tract infections.

Despite intensive treatment, infection of the burns and severe complications such as pneumonia, septicaemia, meningitis and deep vein thrombosis may occur. Diagnosis is hampered by the difficulties of eliciting physical signs in the presence of extensive burns and their dressings. Even in the absence of complications, these patients are very ill, suffering a severe toxaemia, the nature of which is not fully understood. Only the provision of skin cover will lead to improvement and if necessary this may be obtained from a donor. Such homografts will be shed in a few weeks, but in the interim the patient's own healing will continue and the general condition will be better in preparation for further grafting.

Poor general condition is an indication for surgery rather than for its postponement. In particular, chest complications are often improved by tracheobronchial suction during anaesthesia, and operation should not be postponed in anticipation of an improvement in the chest condition.

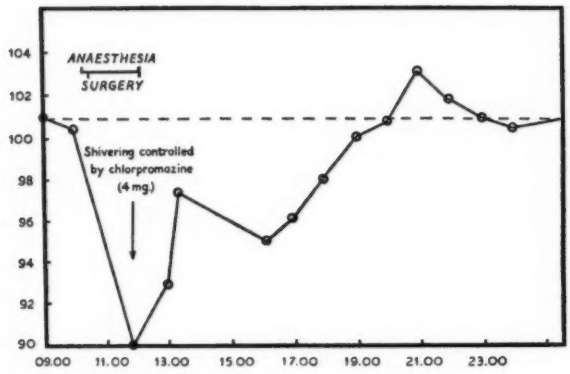
a. Preparation

Oligaemia and anaemia are prevented and treated by transfusion where indicated, and disturbances of electrolyte balance are avoided by a careful study and control of intake and output (Moyer, 1949; Kyle & Wallace, 1950; Bull, 1954; Bull & England, 1954). As a result of these procedures, the pre-operative condition of the patient is at its best.

Great attention is paid to nutrition, and tube feeding is used when necessary. Crasilneck (1955) has reported the successful use of hypnosis to encourage the eating of large quantities. Despite the use of all practicable measures to improve the patient's condition, this sometimes, in the worst cases, remains precarious. The morale may suffer, but the exhibition of sedatives and tranquillizers should be restrained. Premedication will be chosen with care and the dosage of atropine kept low.

Failure of compensating mechanisms is a feature of the severely burned. The ability to adjust the blood pressure to changes in posture is lost (Allen & Slocum, 1952), with the result that severe collapse may occur as a result of moving the patient to apply dressings. Heat regulation is impaired by skin destruction in the burned areas and by the taking of skin for grafting. Profound hypothermia may occur during

FIG. 1. TEMPERATURE RESPONSE TO ANAESTHESIA AND SURGERY



Abscissae: time of day

Ordinates: rectal temperature (°F.)*

Scale at top left of figure shows duration of anaesthesia and surgery

Record of girl aged 5 years; 45% of the surface area of the body burned; 40% full thickness. Débridement and graft

Pre-medication: papaveretum (5.5 mg.) and atropine (0.45 mg.)

Induction: cyclopropane and oxygen

Maintenance: methyl *n*-propyl ether, oxygen and nitrous oxide

* t° F. = $\frac{9}{5}$ (t − 32)° C.—Ed.

surgery, by heat loss from raw surfaces and by blood cooling due to substantial transfusion (Middleton, 1957b; fig. 1 of this paper). There is little doubt that such hypothermia can be turned to advantage in the protection of the patient; chlorpromazine may be given to control shivering or the spastic state which interferes with the spreading and fixation of grafts.

Hyperpyrexia due to skin destruction and to bulky dressings is a common danger in hot weather, especially during the immediate post-operative period, and must be anticipated. The ward, if not air-conditioned, is kept cool by the use of fans. An indwelling rectal thermometer of the dial type permits constant temperature supervision, and sponging of any available skin surfaces is carried out. The use of chlorpromazine in divided doses assists heat loss. Consideration may be given to the use of intra-gastric cooling (Khalil & MacKeith, 1954).

b. Anaesthetic Management

The anaesthetic management of these patients resembles that of primary excisions; but, because of the poor general condition, smaller quantities of the anaesthetic drugs often produce adequate anaesthesia and it is essential to avoid even slight degrees of anoxia.

Curettage of exuberant granulations to produce a suitable bed for the reception of graft is a procedure fraught with risk. These granulating surfaces are usually highly infected (Brown & Altemeier, 1953) with organisms often resistant to antibiotics and are at the same time a source of danger to the patient and to the surgical team. The curettage produces shock out of all proportion to the extent of the procedure, and the anaesthetist should be forewarned. Surgery should not start until the transfusion is assured.

As was the case in primary excisions there are individual preferences. When intubation is necessary (L.J.W.) it is performed after full oxygenation, and relaxation with suxamethonium. In small children, anaesthesia is usually induced by inhalation, commonly with nitrous oxide and trichloroethylene. The drip apparatus is put up when the child is asleep, and intravenous supplements to nitrous-oxide anaesthesia may be given when necessary, as described for primary excisions.

A different technique is favoured by H.G.M. In general all patients undergoing anaesthesia as in-patients are intubated, and the burned are no exception. Not the least advantage is the achievement of a certain dexterity which is valuable in the difficult intubations of those burned about the face and neck and for intubation in the prone position for burns of the back, shoulders and buttocks.

Induction with cyclopropane and oxygen is preferred for infants and the very ill, followed by methyl *n*-propyl ether and oxygen with or without nitrous oxide. Alternatively a sleep dose of thiopentone is followed by an oxygen–nitrous-oxide mixture supplemented by increments of gallamine.

It is a common finding that veins appear after anaesthesia has been induced where none were visible before and for this reason a patient is never subjected to a transfusion immediately before operation.

Guest's cannula (Guest, 1941) is particularly suitable for these patients, and the use of the external jugular vein is frequently desirable. A breath of strong ether can be given to cause straining, which simplifies the venepuncture.

3. Recovery from Anaesthesia

The loss of vasomotor control previously described is guarded against by the minimum of moving and turning when dressings are applied at the end of the operation. With forethought and assistance, most dressings may be applied with but little movement. The use of an orthopaedic operating-table can be of great help in some instances.

Post-operative stridor occurring in these patients has been described by Rook (1953) and by Prendiville & Middleton (1954), and each gave details of three cases requiring tracheotomy. It seemed likely that either the presence of a throat pack or movement of the tube during the application of dressings caused laryngeal trauma of unusual severity in these very ill patients. Disturbances cause much less deterioration when the patient is adequately transfused. An important part of the anaesthetist's function is to assess the blood loss in collaboration with the surgeon, and to replace the blood lost so that no serious degree of oligaemia ever occurs.

The criteria for assessment of blood loss are clinical (Wolfson, 1956; Clarke & Fisher, 1956). Swab weighing and blood-volume estimations have not been found necessary except for primary excisions. A series of 69 cases investigated by Topley & Jackson (1957) shows that this clinical assessment was correct within 20% in 80% of cases. In no case was there gross error of assessment.

Recovery from anaesthesia should be smooth and consciousness regained early.

The transfusion of large quantities of blood during these operations may cause pronounced cyanosis for several hours, due to the effect of the acid-citrate dextrose used as an anticoagulant. It is significant that after transfusion the blood is unable to give up as much oxygen as before although the

haemoglobin content has been raised (Valtis & Kennedy, 1954). Energetic application of oxygen therapy and the early resumption of physiotherapy are of great importance, as is the resumption of feeding. The frequent opening of the oxygen tent must be compensated for by the maintenance of an adequate supply and by the practice of regular analysis (Middleton, 1955).

4. Late Results

A patient sustaining an extensive burn may reappear for surgery many times over a period of years, at first for grafting and subsequently for the repair of contractures or for the transfer of flaps to such areas as the foot or hand. Loss of morale is prevented by a positive attitude of friendliness towards these patients on the part of all grades of hospital personnel. A satisfactory rapport between anaesthetist and patient is enhanced by regular pre- and post-operative visiting and by the use of individually chosen drugs given before operation.

A cumulative anaesthetic record system has been used for the last eight years (Middleton, 1957b). Each patient's response to pre-medication is recorded as a percentage of that which the anaesthetist would regard as perfection. Any shortcomings or overdosage can be allowed for on subsequent occasions. Wide variations are found by this system, and the use of any routine pre-medication becomes inexcusable.

In the restoration of the hand to function after burning, causalgic pain and coldness may be relieved permanently or for long periods by stellate ganglion block and this can be combined with physiotherapy with an advantage which is at once obvious and encouraging to the patient. This has been performed (H.G.M.) on a large number of patients, using, for the most part, a solution of 2% lignocaine injected into the ganglion by the anterior approach described by Davies (1952b).

5. Conclusion

Very much remains to be discovered in the treatment of burns and the role of the anaesthetist must continue to be an important one. He will be faced with many very serious risks which he will accept without delay or demur. He will have to take an active and informed part in a number of ancillary services—transfusion, blood-loss estimation and resuscitation; and he must ensure that no fault in technique loses him the confidence of his patient.

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HYPOTHERMIA IN SURGERY: THE PRESENT POSITION

Including its Influence in the Modern Treatment of Cardiac Arrest

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1 Indications
2 Methods
3 Problems in the management of hypothermia
  a Temperature
  b Anaesthesia
  c Cardiac arrhythmias
4 Treatment of cardiac arrest and ventricular fibrillation
References

Although the present-day technique of deliberately reducing the body-temperature as a therapeutic measure has become wide spread only in recent years, the idea that cold might be beneficial under certain circumstances was first suggested by Robert Boyle in 1772, and twenty odd years later James Currie (1797) attempted to treat the hyperthermia of typhoid fever by immersing patients in ice-cold brine. Before the advent of modern cardiac surgery there had been no stimulus for the use of hypothermia, but interest was aroused when Bigelow and his co-workers (Bigelow, Lindsay, Harrison, Gordon & Greenwood) in 1950 suggested that the reduced oxygen demand of the brain at low temperature would protect it from the inevitable cerebral ischaemia that occurs during intracardiac operations. The first reports of human cases were published in 1952 (Cookson, Neptune & Bailey) and in 1953 (Lewis & Taufic; Swan, Zeavin, Holmes & Montgomery), and by 1955, when the subject was reviewed independently by Delorme (1955) and Churchill-Davidson (1955), hypothermia had become an accepted surgical technique.[1]

1. Indications

Hypothermia is of the greatest value for surgery in which the circulation to part or all of the body has to be occluded deliberately for a definite period of time, hence it is of use for operations on the heart, the brain and the great vessels. In cardiac surgery it has been used during the repair of atrial septal defects and the relief of pulmonary and aortic valve stenosis (Brock, 1956; Swan & Blount, 1956). The repair of ventricular septal defects has also been undertaken, but the results have not been so satisfactory as in the other three conditions. In cranial surgery the successful correction of berry aneurysms has been reported (Botterell, Lougheed, Scott & Vandewater, 1956) and also the excision of intracranial tumours (Lundberg, Nielsen & Nilsson, 1956). In these cases the cerebral circulation was occluded for varying periods of time without any evidence of neurological disturbance afterwards. Carotid artery reconstruction too has been carried out with this technique (Eastcott, Pickering & Rob, 1954). Apart from heart and brain surgery, hypothermia has been used in the repair of aneurysms of the aorta, the reduction in metabolism permitting interruption of the circulation to the lower half of the body for periods of over one hour (Thornton, 1957).

Deliberate cooling has been advocated for other conditions: for example, as an adjunct to anaesthesia in patients who are poor surgical risks (Murray, 1955), in the treatment of acute poliomyelitis with hyperpyrexia and bulbar paralysis (Miörner, Haeger & Ryd, 1955) and for post-partum haemorrhage with irreversible shock (Murray & Bruce, 1955). In most of these, however, the protection afforded by the hypothermia has been assessed by clinical impression only, and it is doubtful whether the technique is justified for such cases.

2. Methods

There is a variety of ways in which the subject can be cooled, but all are basically modifications of two methods, surface or vascular. Of these two different ways, each has its own supporters: to the one group, surface methods are ". . . both aesthetically and surgically unattractive " (Brock, 1956); to the other, vascular cooling is unnecessarily complicated (Gray, 1956). In fact there is no difference in principle —in the one the body is cooled from the skin inwards, in the other from the blood stream outwards, and the only differences are in technical detail and application.

Surface cooling consists of wrapping the patient in a refrigerated blanket (Inglis, Biffen & D'Abreu, 1954), surrounding him with ice-bags (Burrows, Dundee, Francis, Lipton & Sedzimir, 1956), or immersing him in ice-water (Swan *et al.* 1953). It is simple, but may be slow as there is a warm layer of air or water near the subject which acts as an insulator and prevents a rapid fall in temperature. This can be obviated with the immersion method by having the water circulating, so that the patient is no longer in a bath, but in a " river ". A practical difficulty is the handling of the patient, because only during certain types of neurosurgery is it possible to operate on the patient while he is still in the bath, and to move an adult in and out is not easy. However, if the patient is placed in a sling before being put in the bath, he can be lifted more easily, and immersion cooling is the method of choice unless the chest has to be opened during the course of the surgery.[2]

The present-day vascular technique, devised originally by Delorme (1952) and modified by Ross (1954), consists of cannulating the superior and inferior venae cavae via the right atrium and pumping blood mechanically from one to the other through tubing surrounded by a refrigerating medium. Anticoagulants are unnecessary as blood does not clot at low temperature. This method offers advantages for cardiac surgery because the heart can be exposed and the diagnosis confirmed before cooling starts, but it is not practical below the age of three years because of technical difficulties in cannulation. Even from the age of three to ten years, it is

[1] See also Ross, *Brit. med. Bull.* 1955, **11**, 226.—Ed.

[2] See also Delorme, *Brit. med. Bull.* 1955, **11**, 221, and Ross, *Brit. med. Bull.* 1955, **11**, 226.—Ed.

doubtful whether the added technical complication of vascular cooling is worth while, but above that, it is ideal.

Whichever way the patient is cooled there is the problem of where the temperature should be taken. The human being, having a large, weighty structure, cools unevenly, and it is important to record and observe temperatures in several parts of the body, especially those of the two most vital organs, the heart and the brain. It would be ideal if these could be measured directly, but this is impossible clinically. However, a thermometer or thermo-couple placed one-third of the way down the oesophagus is sufficiently near the heart to give an indication of the temperature of that organ, and recordings made at the back of the nasopharynx will indicate the temperature in the base of the brain. Other readings by themselves are unreliable. For example, the rectal temperature lags behind the rest of the body in adults, whereas with immersion cooling in small children it is often ahead. The pharynx has been recommended (Ross, 1956), but it is almost impossible to keep a thermometer or thermo-couple in this position unless packing is used, which will itself modify the temperature. Below 30° C. the skin of the forehead has been found to give a good over-all picture.

3. Problems in the Management of Hypothermia

a. Temperature

How far the temperature should be reduced depends on the length of time it will be necessary to cut off the circulation to the brain. The absolute safe ischaemic time at any temperature has not yet been ascertained. Ross (1956) states that ten to twelve minutes of cerebral ischaemia can be withstood at 28° C., but this temperature was measured in the pharynx; probably that in the brain was lower. The heart of the human adult can be stopped with safety for five minutes if the nasopharyngeal temperature is 28° C. An increase of one-and-one-half minutes may be permitted for every further fall of one degree (Lucas, 1957). But there are modifying factors: the young are more resistant than the old, and the state of the brain immediately before the circulatory occlusion is important.

b. Anaesthesia

The essential feature of the anaesthetic technique is to use minimal amounts of drugs (Lucas, 1956). It is necessary to control shivering, but there is general agreement that the relaxants are preferable to deep anaesthesia for this. As the temperature falls, the effect of the anaesthetic agent is increased, hence less is required; but it cannot be dispensed with altogether as, unlike some animals, man can be conscious at 26°–27° C. Short-acting agents are best, and drugs such as pethidine or chlorpromazine should be avoided, although this latter drug was popular at one time for hypothermia because it is a marked vasodilator and so was thought to speed cooling. Sellick (1957) has recommended the use of ether in order to protect the heart against cardiac arrhythmias, but the evidence is not particularly convincing.

The most important part of the technique is to overventilate the patient deliberately throughout the procedure to prevent the occurrence of a respiratory acidosis. Cardiac irritability is known to be more marked in the acidotic state at normal temperatures (Johnstone, 1950) and there is some evidence that this is so at low temperatures (Osborn, 1953). Clinically there is no doubt that inadequate ventilation increases the incidence of cardiac arrhythmias, but this is difficult to prove experimentally in so far as pH measurements at low temperature cannot be correlated accurately with those at normal temperature.

c. Cardiac Arrhythmias

A great variety of cardiac arrhythmias have been described under hypothermia, but not all are significant. Cooling is usually accompanied by a slowing of heart rate, depression of intracardiac conduction, and inhibition of the normal centres of impulse formation with resultant atrial fibrillation (Hicks, McCord & Blount, 1956). The disturbance in conduction becomes manifest at temperatures below 33° C. and is of little importance, although Fleming & Muir (1957) have suggested that those patients who develop atrial fibrillation early are less likely to develop ventricular fibrillation. Below 30° C. ventricular arrhythmias appear and at one time were thought to predispose to ventricular fibrillation. There was supposed to be a " fibrillatory zone " between 28° C. and 24° C. and for this reason deep hypothermia was considered dangerous. It is now known that ventricular fibrillation can occur at any temperature and that it is related to disturbances of the metabolism of the heart. There are four precipitating causes: deep anaesthesia, respiratory acidosis, mechanical irritation to the heart, and a poor coronary flow. The first three are avoidable, but the last is dependent to some extent upon the state of the heart. As the body-temperature falls, the cardiac output drops, but in the early stages there is a marked vasoconstriction which maintains the systemic blood pressure at a level compatible with adequate coronary flow. However, at about 28° C. the vasoconstriction can no longer compensate for the reduced cardiac output, resulting in a low blood pressure with an ineffective coronary circulation. Hearts which have been in cardiac failure previously, and whose myocardium is impaired, may show this reduction in cardiac output at a much higher temperature. Temporary relief can be afforded by the use of pressor agents such as noradrenaline (Brock & Ross, 1955), but these are difficult to control. Prostigmine (Montgomery, Prevedel & Swan, 1954), procaine amide (Wulff, Haeger & Ryd, 1955), and a host of other drugs have been suggested as a means of preventing ventricular fibrillation in the cold state, as has infiltration of the sino-atrial node with procaine (Riberi, Shumacker, Kajikuri, Grice & Boone, 1955); but in clinical practice they have not proved satisfactory.

When ventricular fibrillation does occur it is possible to treat the complication without haste because of the decreased oxygen demand brought about by the low temperature. In open-heart surgery there are often occasions when the heart has to be re-started, following the period of circulatory occlusion. As a result it has been possible to make a systematic study of cardiac arrest and ventricular fibrillation and their appropriate treatment. This treatment can be applied equally well at ordinary temperature.

4. Treatment of Cardiac Arrest and Ventricular Fibrillation

The essential feature in the treatment of cardiac arrest or of ventricular fibrillation is to re-establish an adequate cerebral circulation; then measures can be taken to restore an effective beat. These are two separate problems and must be treated as such. The first demands immediate cardiac massage and

oxygenation of the lungs. Time is the important factor, otherwise irreparable neurological damage may ensue; and from the moment it has been decided that the heart has stopped, vigorous cardiac massage must be started and the patient given artificial respiration, through an endotracheal tube if necessary. Time must not be wasted in injecting drugs either into the heart or elsewhere, as none of them has ever been shown to be 100% efficient in re-starting the heart. Cardiac arrest can occur reflexly under certain conditions of mild anoxia and vagal over-activity (Sloan, 1950), and in such cases the heart will re-start with any stimulus, even the prick of a needle. As a result the occasional success with the use of drugs in this type of arrest has led to a false impression of their value.

The heart can be massaged either through the chest, via an anterior incision in the fourth intercostal space, or through the abdomen. The former is preferable because it enables the operator to obtain a better grasp of the heart and, if the pericardium is also opened, the heart can be observed directly. The abdominal route should be used only if the cardiac arrest occurs during an abdominal operation, and, even then, if massage is not effective within one minute, the abdominal route must be abandoned in favour of the thoracic route. Cardiac massage must produce a carotid pulse. The rate is unimportant—obviously the quicker the better—but time must be allowed between squeezes for the heart to fill. In order to ensure the maximum blood flow to the brain, arterial tourniquets can be placed around all four limbs, but this must not be done until after massage has been started.

Once there is evidence that oxygenated blood is reaching the brain, as seen by a change of colour in the face and a reduction in the size of the pupils, attempts can be made to re-start the heart. Before any heart will start beating spontaneously it must have a good muscle tone. This can be achieved by improving the coronary circulation, i.e., by stronger massage. Obstructing the aorta for a few seconds above the origin of the coronary vessels is helpful and, if the arrest has occurred during a thoracic operation, it may be possible to use an intra-aortic transfusion to boost the coronary circulation. Once cardiac tone has returned, the heart will probably re-start of its own accord, but if not the tone can be improved still further by the use of 5–10 ml. of 1% calcium chloride, or 5–10 ml. of a 1 : 10,000 solution of adrenaline. These drugs should be injected into the left ventricle, and massage continued. They should not be given together and should be repeated only after an interval of 10 minutes.

The treatment of ventricular fibrillation is the same as that for asystole, but it is even more important that massage should be continued until cardiac tone returns; otherwise defibrillation will never be successful. The tone can be estimated from the fact that atonic ventricular fibrillation is fast and fine, whereas the fibrillation will be slow and coarse when tone returns. Once the heart is fibrillating slowly it can be defibrillated electrically by an alternating current of up to 200 v, passing 1.5 A for one-fifth of a second. If this is not immediately successful, it can be repeated, but before the second shock 5 ml. of 1 : 10,000 solution of adrenaline should be injected into the left ventricle. Electrical defibrillation can be repeated as often as is necessary, massage being continued in between, but caution should be used in repeating the adrenaline injection. Sometimes the heart reverts spontaneously to a normal beat on return of tone, and electrical defibrillation is unnecessary.

How long cardiac resuscitation should be continued depends on whether the heart and brain are receiving an adequate supply of oxygenated blood. If this is achieved, massage may have to be continued for some time to enable the drugged, post-anoxic heart to recover and resume beating; a successful outcome has been reported after as long as two hours of massage and electrical defibrillation (Milstein, 1956). However, if the cerebral circulation is inadequate, as evinced by widely dilated pupils which do not constrict on even more vigorous massage, resuscitation can be abandoned after 15 minutes. Commonly, in a fatal case, cardiac massage appears to be effective for a period of about 30 minutes and then quite suddenly the peripheral circulation fails and an adequate cerebral and coronary blood flow can no longer be obtained. Even when resuscitation is successful, the wound should not be closed for at least 30 minutes because the heart may stop again. More than one apparently successful case has been lost because the patient has been returned to bed too soon.

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THE ADVANTAGES OF CONTROLLED HYPOTENSION IN SURGERY

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1 Surgical advantages
2 Hypotensive technique
3 General observations
References

The use of hypotension to minimize bleeding in surgery has been criticized by anaesthetists opposed to the principle of physiological trespass in anaesthesia (Davison, 1953). But even the most adverse critics admit the practical possibilities of a reduced blood pressure in certain operations, and the advantages which can be obtained in this way, and that the technique may be justified in some fields of surgery (Little, 1956). At the same time it is considered unsafe because accidents have occurred, and, to explain them, all the hazards of uncontrolled hypotension have been advanced. It is indeed essential to know the possible hazards associated with hypotension, but also to realize that only careful research will indicate their importance and that speculation and academic argument do not assist materially. That the reduction in blood supply to the brain in the reversed Trendelenburg position can be dangerous is not even questioned. That irreversible brain damage occurs with circulatory arrest is known only too well, and great care must be taken to watch the heart's action continuously during hypotension. *Un*controlled hypotension is undoubtedly extremely dangerous and merits the adjective "deadly". On the other hand a carefully induced and controlled hypotension can now be accepted as a safe and reliable technique in surgery. The present communication is not compounded from the numerous reports in the literature of other workers' findings, interesting as they are, but is concerned entirely with the practical experience which I have gained with hypotension during the last eight years. A technique has been built up slowly during this time, in which certain outstanding essentials to safety are recognized that have enabled a large amount of work to be completed satisfactorily and safely. My observations are based entirely on this work, and are concerned more especially with plastic and reconstructive surgery, together with some general surgery and surgery of the ear, nose and throat.

1. Surgical Advantages

The surgical advantages have been stated clearly by McIndoe (1956). He says:

> Few surgeons would disagree with the dictum that every operation is a gross injury to the tissues of the body, limited only by the delicacy and care with which it is performed.
>
> At a conservative estimate, 50% of a surgeon's time and a great deal of his nervous energy are devoted to controlling bleeding. The rest is concerned with the real object of the operation. This is especially true in operations of difficulty or of great extent. Now that postoperative sepsis is rare, most of his postoperative anxieties are due to reactionary hemorrhage or to problems arising from the damage done by his efforts to control bleeding during the operation. The bruising effect of repeated swabbing upon the tissues, the crushing effect of clamps and the multiple points of possible sepsis and certain fibrosis caused by dozens of ligatures all make the difference between a first class and a mediocre result.
>
> How many plastic surgeons have, after an anxious time-consuming operation, troublesome with persistent bleeding, looked with dismay at the flap, pedicle or free graft bulging with blood some hours after operation and realised that the prognosis for a good result, whatever is done, is now a poor one!
>
> Most mistakes in surgery arise from not seeing rather than from not knowing and a bloody field during operation is equal only to inadequate exposure as a producer of surgical accidents and mistakes.

McIndoe then proceeds to a more detailed analysis of the surgical advantages as follows:

> From the surgeon's point of view the opportunities offered by a bloodless field are indeed so great as to constitute a major surgical advance. Not infrequently, controlled hypotension affects not only the success of the operation but the chance of survival of the patient. Generally speaking, however, it maintains a clarity of vision in difficult inaccessible regions so that the operation is more exact, more quickly performed and thereby more successful. Thus the scope and extent of surgical operations in certain areas is widened and the operative time reduced.

These advantages are demonstrated clearly in the operation for secondary cleft lip repair where three stages are now combined into one operation. Or again, in dissecting small delicate structures such as the lachrymal apparatus, clear visibility is all-important if such procedures as dacryocystorhinostomy or end-to-end anastomosis of canaliculi are to be successfully carried out. Massive haemorrhage is no longer feared in certain operations on the head and neck—for example, excision of massive facial cavernous naevi or arteriovenous aneurysms. And post-operatively there is a striking reduction in the incidence of haematomata, provided that the return of pressure is carefully controlled. Indeed practically all major operations on the head and neck—such as block dissection of the glands of the neck, forehead rhinoplasty, laryngectomy, external ethmoidectomy—are now conducted under this technique. Grafts can be applied to a dry field untouched except for the initial sweep of the scalpel. Hand operations (tendon graft, nerve anastomosis, clearance for Dupuytren's contracture, or other procedure) are greatly facilitated by the dry field afforded by hypotension and elevation of the hand after the tourniquet is released. Operations on the chest wall such as radical mastectomy can be performed with minimal blood loss and no shock to the patient. Wherever the site of operation can be elevated above heart level, some considerable advantage can be obtained. This cannot be achieved easily in the upper abdomen, or for operations on the body wall at heart level. Elsewhere some degree of postural ischaemia can always be obtained, except in the perineum when the legs are elevated, as in the lithotomy position. It is important to stress the advantages of postural ischaemia when employing hypotension, also the importance of co-operation between anaesthetist and surgeon so as to achieve the best results. Indeed, in most fields of surgery there is often the opportunity to obtain many benefits by the skilful use of controlled hypotension, in close liaison with the surgeon.

2. Hypotensive Technique

These surgical advantages are definite and real and enable more satisfactory results to be obtained with considerable advantage to the patient. There can be no doubt whatsoever about the desirability of hypotension nor of its advantages in certain operations; but it must be achieved with safety, otherwise all these advantages are lost.

Gillies (personal communication, 1949) postulated that a systolic blood pressure of 60 mm. Hg was adequate for cellular respiration and metabolism, provided that vasodilatation and full oxygenation were ensured. This pressure at heart level has been accepted as a minimum, and to it has been added the postural ischaemia of an elevated operation site (Enderby, 1950). From the early investigations into the effects of ganglion-blocking drugs, it was at once apparent, however, that hypotension could be achieved only by a postural limitation of venous return to the heart, as in the reversed Trendelenburg position (Enderby, 1950). Postural hypotension is the basis of the technique today, but considerably greater knowledge has been obtained in the meantime, enabling blood pressure to be controlled more easily and steadily. At first it was possible to achieve an adequate reduction of blood pressure in only a few patients, mainly those with hypertension, and certainly the course of the hypotension was erratic and very often uncontrolled. During the intervening years the various factors affecting blood pressure have been carefully studied and observed, and as a result it is now possible to obtain a more carefully controlled hypotension within strictly defined limits, and with due regard to certain safety signs (Enderby, 1956).

The effects of all ganglion-blocking drugs vary considerably from patient to patient. When the initial response to the drug has been assessed, it is essential to know by what means the blood pressure can be maintained at this level if adequately low, or how it can be reduced further to the required level, and then maintained for the duration of the operation with a safe—and sure—return afterwards. The safety and success of the whole technique of controlled hypotension depends on being able to do this whenever necessary. As our knowledge of the different mechanisms which control blood pressure has increased, so have the success and safety of this technique. It is undoubtedly true that the practical difficulties of controlling blood pressure carefully and accurately in each patient are very considerable. Indeed the major difficulty facing any anaesthetist who employs this technique is the great variability of patients, and the judgement and skill necessary to obtain the initial fall of pressure to a safe level, and to maintain it at that level for the duration of the operation. If it had proved possible from the start to control blood pressure easily and carefully there would have been fewer adverse critics today, and the accidents consequent on uncontrolled hypotension would not have occurred.

Control in Hypotensive Techniques

All anaesthetists skilled in the control of blood pressure admit the vital necessity in this technique of good anaesthesia with full sensory blockade and muscular relaxation. Furthermore, too much care cannot be given to the airway and to full oxygenation, both of which must be beyond suspicion all the time. Ganglion paralysis is induced by some suitable intravenous drug; there is now a large number of these drugs from which to choose. My preference is for the long action of pentolinium for the greater part of my work, with Arfonad on those occasions where the duration of hypotension is especially short, as, for example, after release of an exsanguinating tourniquet on the arm. The full effect of the ganglion paralysis is observed with the patient in the horizontal position first, and this requires 3–5 minutes to develop.

It is most important to realize that the control of blood pressure from this stage is entirely under the supervision of the anaesthetist. The extent of the fall in pressure and its duration depend only to a small degree on the particular ganglion-blocking drug used and on which anaesthetic and relaxant drugs are employed, and almost entirely on certain physical effects which are exerted on the patient thereafter. Thus, when the body is tipped into the reversed Trendelenburg position there is a reduction in the venous return to the heart by gravitational pooling of blood into the dependent parts, and consequently the blood pressure falls to a low level. At the same time a gradient of pressure is established throughout the body, as a result of which the elevated regions are reduced in pressure below that at heart level by 2 mm. Hg for each 25 mm. (1 inch) of vertical height, whilst the dependent parts are elevated in pressure by a similar amount. The combination of a low systolic blood pressure with a further reduction of local blood pressure in this manner results in a postural ischaemia, and explains the local pallor and reduction of bleeding achieved by this technique.

Postural hypotension obtained in this way is extremely unstable. The initial fall may be severe and produce a dangerous hypotension, or on the other hand it may prove inadequate. The extent and duration of the fall depend in large part on the pre-existing balance between the sympathetic and parasympathetic nerves of the body, and the almost certain persistence of some autonomic reflexes, even after ganglion paralysis which, at its best, is only a partial block. In young healthy patients profound postural hypotension does not last for longer than 5–15 minutes, after which time it must be augmented by the skilful use of controlled respiration if it is to be maintained. When respiration is controlled and its normal "suction-and-force-pump" action is replaced by positive pressure insufflation there is no longer any assistance to the venous return to the heart, but instead interference caused by direct pressure on the great veins and pulmonary circulation. Consequently instead of active assistance to the venous return there is an active restriction as the lungs and great veins are squeezed against the relatively resistant chest wall, diaphragm and mediastinum. The hypotensive effect of this is marked, especially during the immediate period following the administration of ganglion-blocking drugs, and must be used with great care, moderation and skill; then it is of considerable value in maintaining a steady hypotension.

A further vital influence of respiration on blood pressure is the effective excretion of CO_2; this is essential for control of blood pressure and maintenance of hypotension. Carbon dioxide exerts a very powerful influence over muscle tone and venous return to the heart. It is not surprising, therefore, that controlled hypotension becomes much easier when the CO_2 level is maintained at a minimum. The efficiency of the anaesthetic machinery in removing or absorbing CO_2 is very important. Acapnia induced by hyperventilation assists in maintaining the initial fall of pressure, whilst a rise in the CO_2 level soon initiates reflexes which rapidly return the pressure to normal. These reflexes are probably mediated through a

secretion of adrenaline. Because of the fine adjustment of blood pressure which can be exercised by respiratory control, CO_2 absorption and intrapulmonary pressure, it becomes clear why a closed-circuit apparatus is favoured. To employ this technique with skill and accuracy it is almost essential. An entirely closed technique can be used, or the gas flow can be increased and allowed to spill over through the valve if desired. A circle-type apparatus with to-and-fro absorption has proved entirely satisfactory in my own practice, but the absorber must be of good design so that the CO_2 is absorbed fully.

In many patients a controlled hypotension can be maintained successfully only by an adequate postural and respiratory control, which is achieved by full CO_2 excretion, and by the effects of controlled respiration on venous return to the heart and on the pulmonary circulation. It must be remembered, however, that it may be unnecessary to employ all these controls in every patient, and, particularly in the older ones, natural respiration may not elevate the pressure above the safe minimum. There are many different ways in which the three variables of posture, respiration and CO_2 absorption can be employed to achieve the most satisfactory control in any particular patient, whilst at the same time maintaining the patient in a position suitable for the surgeon. All these controls are in the hand of the anaesthetist who, by skilful controlled respiration and positioning of the patient, can maintain hypotension at the required level.

3. General Observations

This technique has been evolved gradually over the past eight years, during which time a considerable number of patients have been treated by it (2,800 by myself, and a total of 6,000 with other members of the staff of the Queen Victoria Hospital, East Grinstead). During this time there has been a gradual progression along the path of "physiological trespass" in order to prolong control and render it more accurate. This has at the same time produced a greater understanding of the various factors affecting the control of blood pressure and of how they can be modified. It is of course unnecessary for me to add that all "trespass" must be employed for only as short a time as possible, and then only when all other conditions are satisfactory. The indications and contra-indications cannot be discussed here, but it can be stated that ill effects have not been observed from these techniques. Varying degrees of positive-pressure respiration have been employed in about 500 patients. Full CO_2 excretion with hyperventilation, the value of which was appreciated only more recently, has been practised in about 250 patients. These techniques are only aids which the anaesthetist employs to help obtain and maintain a controlled hypotension. They are potentially dangerous because they enable blood pressure to be depressed to a level at which damage to heart and brain can occur. In this total of 6,000 cases, cardiac arrest occurred in four patients, in one of whom there was full recovery. Two of these were among the first 250, and their cases have been fully described elsewhere (Enderby, 1952). In all these catastrophes there was clear evidence that the cardiac arrest was caused by inadequate oxygenation. They are therefore avoidable, and they point clearly and distinctly to the vital necessity of maintaining an adequate airway and full oxygenation at all times during and after operation, and more especially so when ganglion-blocking drugs have been employed. They bear out the observation that the myocardium will not tolerate hypotension below 40–60 mm. Hg, particularly if it is associated with suboxygenation. The immediate danger of hypotension is to the myocardium, but the brain may suffer damage in the head-up position unless extreme care is taken to maintain the minimum pressure. In the same series there were two instances of cerebral thrombosis, one caused by a period of excessive hypotension during a difficult and prolonged operation on a malignant growth of the mandible; the other occurred 12–16 hours after full recovery from an extensive breast operation. Other complications, such as renal anoxia, hepatic damage, and paralytic ileus, have not been encountered in this large series. This would appear to be very reasonable evidence in support of the safety of this technique, and an indication that the theoretical dangers, although they cannot be ignored, are nevertheless of minor importance when control is fully exercised. If this were not so, the technique of controlled hypotension would not be practised as it is today.

As a result of our experiences with hypotension, it can be stated with absolute confidence that a period of hypotension can be induced safely and satisfactorily in the majority of patients for the duration of an average operation, but special attention must always be given to the airway and oxygenation, and the blood pressure must be carefully watched and maintained at a minimum of 60 mm. Hg at heart level, particularly when the head is elevated in the reversed Trendelenburg position. The complications which have been encountered have only emphasized the vital necessity of skilful control in the conduct of this technique, which when practised within strict limits is a safe procedure.

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THE DISADVANTAGES OF CONTROLLED HYPOTENSION IN SURGERY

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1 Haemodynamics
2 Incidence of complications
3 Contra-indications
References

In the light of previous work on the control of haemorrhage during surgery by lowering the blood pressure (Gardner, 1946; Griffiths & Gillies, 1948), the hypotensive action of pentamethonium was quickly seized on when this drug was first introduced as an antidote to decamethonium (Davison, 1950; Enderby, 1950). Although there were some who greeted this development with enthusiasm, from the first I had misgivings (Davison, 1950, 1951, 1952a, 1952b, 1953), which are confirmed in a recent comprehensive work (Little, 1956); and no one will now deny that all methods of induced hypotension carry risks greater than those associated with "orthodox" anaesthesia.

1. Haemodynamics

The life of each part of the human body depends upon its blood supply. Cessation of blood flow necessarily leads to the death of the part concerned after a period of time which varies from a few minutes to a few hours, according to the metabolic needs of the tissues. Obviously, if the surgeon is to be given a completely bloodless field for operation, the circulation through that part must be at a stand-still; equally obviously, when ischaemia is obtained by hypotension and posture, adjacent parts not necessarily involved in the operation also suffer from deprivation of blood (e.g., the brain during fenestration operations).

There are several ways of securing a bloodless field: e.g., by tourniquet or by the infiltration of a vasoconstrictor; induced hypotension is one of these methods. The blood pressure is, of course, only indirectly connected with the blood flow. If, for example, it were possible to lower the peripheral resistance without at the same time increasing the volume of the vascular bed, the blood pressure might fall without any alteration in the rate of flow, or, if the pressure remained the same, the flow would increase. Since a lowered peripheral resistance may involve an increase in the size of the vascular bed, there is often a consequent decrease in venous return to the heart, with a diminished heart size and a decrease in cardiac output.

When the blood pressure falls, the effect of gravity becomes of more importance in determining the path taken by arterial blood through the body, since a column of blood 10 in.[1] in height represents a pressure of 20 mm. Hg. With systolic pressures of the order of 150 mm. Hg, the effect of gravity is insignificant; but, as the pressure falls, this factor becomes more important, so that, eventually, by suitable positioning of the subject, any part can be deprived of its blood supply simply by elevating it.

Unfortunately, the effect of elevation is not exactly proportional to the height to which the part is raised above the heart, and the flow of blood through a part may cease when there is still a considerable head of pressure. One attractive theory, not yet completely accepted, to explain this phenomenon is that the smaller vessels close completely when the pressure falls below the critical closing pressure (Burton, 1952). Evidence of the actual pressures involved is scant; in the frontal lobes of the cat, the blood flow appears to cease at about 20 mm. Hg (Carlyle & Grayson, 1956), but in certain human tissues the necessary pressure appears to be higher, about 50 mm. Hg (Pask, 1955), and there is some electroencephalographic evidence that a pressure of between 45 mm. and 70 mm. Hg is required to maintain the circulation in the monkey's brain (Corday, Rothenberg & Putnam, 1953).

The relevant pressure in the brain of man is not yet known. It is likely that there are considerable individual variations, with the probability of a raised value in patients with hypertension. On the basis of the evidence in the monkey, the normal is probably between 45 mm. and 70 mm. Hg, and it must be emphasized that this is the pressure required in the brain: when the patient's head is raised above the heart, the pressure at heart level must be maintained at a level greater than this to overcome the hydrostatic pressure (20 mm. Hg for 10 in. vertical height). As Pask (1955) has pointed out, this "all-or-none" mechanism might account for the relative absence of minor, but yet the unpredictable occurrence of major, complications after induced hypotension.

The studies by Nilsson (1953) and Nilsson & Vinnars (1957) show that cerebral disturbance is a common sequel to hypotensive anaesthesia, whether the patient be in a head-up or a head-down position. It should be borne in mind that the head-down position, while perhaps less dangerous than the head-up position, nevertheless is not innocent. On account of the large arteriovenous connexions in the neck (e.g., in the thyroid gland), relative stagnation of blood in the brain may occur when there is generalized vasodilatation. Furthermore, it has been pointed out by Aserman (1953) that, in neurosurgical cases, hypotensive anaesthesia predisposes the brain to damage from retractors, owing to its altered consistency.

Reference has already been made to the decreased venous return to the heart which may accompany induced hypotension. Furthermore, a lowering of the diastolic blood pressure leads to a decrease in the perfusion of blood through the coronary vessels. It is obvious that, in these conditions, the heart is not working to its best advantage, and a heart which is already imperilled by disease may well fail completely. As long ago as 1951, I demonstrated a depressed S-T segment in the electrocardiogram during hypotensive anaesthesia, and this finding has been confirmed (Grob, Scarborough, Kattus & Langford, 1953).

It is well known that a fall in systolic blood pressure to about 80 mm. Hg initiates a vasoconstrictive response in the kidneys. It seems to be true that, with the drugs and techniques employed in hypotensive anaesthesia, this response is diminished, yet nevertheless it does occur, especially when there is pre-existent renal damage, which may, however, easily escape pre-operative recognition. The effect of induced hypotension on renal haemodynamics is described by Moyer & McConn (1956) and Moyer, McConn & Morris (1955).

[1] 1 inch = 2.54 cm.—Ed.

Since the drugs commonly employed to produce hypotension are parasympathomimetic in action, there is a risk of bronchospasm, especially in those who have already a disposition in this direction, and two deaths have been reported from this cause (Schwartz, 1949; Davison, 1951).

2. Incidence of Complications

The results achieved with induced hypotension have been set out in great detail by Little (1956), who presents an impressive catalogue of the dangers of hypotension. In response to a questionnaire, Little received reports of 27,930 cases (21,125 in Great Britain and Ireland; 6,805 in the United States of America). The complications recorded include anuria, cerebral thrombosis, coronary thrombosis, cardiac arrest, cardiovascular collapse, reactionary haemorrhage, persistent hypotension and delayed awakening. The total incidence of complications was 1 in 31. There was a highly significant increase in the complication rate when the systolic blood pressure was allowed to fall below 80 mm. Hg. Hampton & Little (1953) had already shown that, in a series of 5,735 cases, the complication rate was only 1 in 127 if the blood pressure did not fall below 80 mm. Hg, but that it was as high as 1 in 15.5 when pressures below this were achieved. It must be remembered that most workers are agreed that the pressure needs to fall to at least this level, if satisfactory reduction in blood is to be achieved. The over-all mortality in Little's 1956 series was 1 in 291; surely, these figures are enough to give pause to the boldest.

Before drawing any conclusions from the foregoing facts, it is worth while considering two great benefits that have arisen from the introduction of the methonium drugs and those with similar pharmacological action; first, the introduction of these drugs into the treatment of chronic hypertension, with inestimable relief to thousands of people, and, with less dramatic results, into the treatment of such conditions as toxaemia of pregnancy and acute ventricular failure; and secondly, the extension of knowledge of the physiology of the human circulatory system which has resulted from the use of these drugs and the intense investigation of their effects. These two far-reaching events are the direct result of the enterprise of British anaesthetists, and, whatever the ultimate fate of induced hypotension in anaesthesia, are positive benefits which are not to be forgotten.

3. Contra-Indications

The extension of the knowledge of physiology has brought to light the fact that the conclusions based upon previous investigation of the circulatory system were indeed correct; no revolutionary new principles have been revealed, and the importance of the blood pressure and blood flow in maintaining the life of the organism is shown to be as great as ever. The blood pressure can be lowered and haemorrhage reduced, but a low blood pressure carries with it an increased risk of grave damage or death to the patient. Is such a procedure ever justifiable? The answer seems to be, "Yes"; but only in certain rather rare cases. Absolute contra-indications to induced hypotension are hypertension, and renal, hepatic and cardiac disease except, perhaps, in ventricular failure. It is only with difficulty that the existence of some of these diseases can be excluded pre-operatively. The relative indications are the importance of preventing haemorrhage and the necessity for operation. If the operation is the only means of saving the patient's life or of removing a symptom so distressing as to make the patient's life unbearable, and if the presence of haemorrhage is likely to render the operation impossible or unsuccessful, then induced hypotension may be considered. It may also be occasionally justifiable in certain cases when blood transfusion is likely to be required but, owing to some rare agglutinin in the patient's blood, is impracticable. It seems reasonable to suppose that, during hypothermia, the risks of hypotension are reduced.

It must never be forgotten that the patient under the influence of methonium and similar drugs has lost the ability to respond normally in certain situations: a slight overdose of, say, thiopentone, a slight accumulation of carbon dioxide as a result of mildly inadequate ventilation or a short period of anoxia from, say, accidental endobronchial intubation, may be tolerated without ill result in the normal patient, but may well spell disaster to the hypotensive person. In effect, the hypotensive patient is on the very brink of the precipice: one stumble, and he will be precipitated into the abyss; there is no room for manoeuvre.

Equally, it must be remembered that, when inducing hypotension, one is gambling against the unknown, and, because of the likelihood of individual variations, probably the unknowable. If hypotension is to secure haemostasis, there is always the probability that, whatever the posture of the patient, the head of pressure in some vital organ will fall below that which is necessary to maintain a flow of blood: the gamble is that the flow will be resumed before permanent damage will have occurred.

Therefore, it can be stated categorically that it is unjustifiable to induce hypotension in the presence of the absolute contra-indications or in the absence of compelling reasons. To use this method during routine herniorrhaphy or the removal of sebaceous cysts is to court unnecessary disaster. The anaesthetist must surely be more concerned with the ultimate welfare of his patients than with simply providing pleasant operating conditions for the surgeon; he must remember the dictum of the physicians of an earlier age: *Primum non nocere*.

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THE TREATMENT OF RESPIRATORY INADEQUACY

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1 Factors conducing to respiratory inadequacy
2 Assessment of ventilation
3 Poliomyelitis
4 Tetanus
5 Narcotic poisoning
References

One of the most important functions of external respiration is the maintenance, within narrow limits, of the proper tensions of oxygen and of carbon dioxide in the arterial blood.

1. Factors Conducing to Respiratory Inadequacy

Respiration is controlled by one of the many homeostatic systems with which all animals are supplied. If this system is upset, respiratory inadequacy results. The system follows the usual plan in consisting of sensing elements, an afferent nervous pathway, a central governor, an efferent nervous pathway, and an effector mechanism. Respiratory inadequacy will result from failure of any of these elements.

The most important sensing elements are in the walls of arteries—including those perfusing the brain-stem. They are affected by the tension of CO_2 ($Paco_2$) and of oxygen (Pao_2), and by the reaction (pH) of the blood flowing over them.

The central governor, in the brain-stem, acts on the information brought to it along the afferent nervous pathways from the sensing elements. It varies the synchrony, rapidity and duration of the stream of impulses which it is continually sending down the efferent pathway to the effector apparatus. At the neuromuscular junction these variations are translated into corresponding variations in the amount of effector substance released, and so in the strength of the resulting contractions of the respiratory muscles.

The minute volume of ventilation is thus adjusted to oppose any tendency of the $Paco_2$, Pao_2, or pH to wander from their normal values. Such a homeostatic system is common as part of " process control " in modern industry; and there is a close analogy between the control system evolved by nature and those invented, in emulation, by man.

The system may be upset by failure of the central control element, by a breakdown in the efferent nervous pathways, by changes at the neuromuscular junction, or by processes—mechanical or pathological—which interfere with the ability of the thorax to present enough fresh air to the pulmonary capillaries. So far as is known, corresponding changes on the afferent side—a breakdown in the receptor organs, in the recepto-neural junction, or in the afferent nervous pathway—occur only as part of a severe generalized malady.

A common cause of respiratory inadequacy is failure of the central governor. It is composed of highly specialized nerve-cells whose function is easily depressed by narcotics and other poisons. It may also be put out of action by trauma. Another cause is a breakdown in the efferent nervous pathway. This may occur, for instance, as a result of anterior poliomyelitis. Pathological processes are often not confined to a single element. Thus poliomyelitis may affect the central control and the effector mechanism, and the same is true of tetanus. The neuromuscular link may be deranged by the relaxants, by certain poisons, and by myasthenia gravis. Finally the effector mechanism—the ability of the thorax to act as a bellows—may be impaired by injury, by pathological changes or by obstruction. This article considers only respiratory inadequacy due to poliomyelitis, to tetanus, and to narcotics.

2. Assessment of Ventilation

It is obviously desirable, in dealing with respiratory inadequacy, to be able to assess its degree, and to know how far the patient's existing ventilation falls short of what is necessary to maintain homeostasis. Information about this may be obtained by comparing the patient's minute volume with what it ought to be, by measuring the oxygen saturation of his arterial blood, or by estimating its P_{CO_2}.

Of course, gross inadequacy is apparent without any measurements and requires immediate treatment; but when some ventilation has been re-established, either spontaneously or artificially, it is important to know whether it is too much, too little, or correct.

The easiest measurement to make is that of the minute volume. It may be measured by a spirometer or by a gas-meter. A spirometer is normally connected directly to the patient's airway, so that the bell rises and falls with respiration, and he rebreathes from it. If more than a few breaths are to be measured, a soda-lime canister must be interposed or CO_2 accumulation may affect the result. Alternatively, non-return valves may be interposed between the spirometer and the patient, so that the instrument records only one phase of respiration. For normal adult tidal volumes the capacity of the bell will allow only a few breaths to be recorded with this arrangement, but when tidal volumes are much reduced it may take a minute or more to fill, or empty, the bell. Gas-meters are of two types: displacement and inferential. In a wet displacement meter, expired gas displaces water successively from a number of chambers mounted radially on a spindle. Each displacement causes a given rotation of the spindle, which is connected to a dial. In a dry displacement meter (the type of gas-meter in domestic use) the gas is diverted by a slide-valve alternately into each of a pair of bellows. Each movement of the slide-valve—corresponding to a known volume of gas—moves a needle over a dial. In inferential meters, the gas stream causes a vane or propeller to revolve, and the volume passed is inferred from the number of revolutions. The best known of these are the American Bennett ventilation meter and the German meter made by Dräger. The Wright Jet Anemometer, developed more recently in Britain, has just become available. The minute volume, obtained by measuring the volume passed into one of these instruments in a given time, must then be compared with the normal for the patient. Normal values can be derived, with sufficient accuracy for most purposes, from Radford's nomogram (Radford, Ferris & Kriete, 1954).

A rough indication of the Pa_{O_2} may often be obtained from the patient's appearance. Cyanosis, however, is an unreliable guide, because of subjective errors (Comroe & Botelho, 1947) or alterations in haemoglobin. The measurement of arterial oxygen saturation is a laboratory procedure. An approximation may be obtained by oximetry, by which the colour of the " arterialized " blood in the capillary vessels of the lobe of the ear or the skin of the forehead is analysed photometrically. Oximeters, however, are tricky instruments. They must be thoroughly understood to give reliable results.

The estimation of Pa_{CO_2} is of more interest and importance. It is more important because, in most cases, a level of ventilation which will ensure carbon-dioxide homeostasis will be more than enough to prevent anoxia. The direct measurement of Pa_{CO_2}, by tonometry, is difficult. Its estimation, by the application of the Henderson-Hasselbalch equation to pH and plasma CO_2 content, is not difficult but requires the resources of a competent biochemical laboratory. Another way of finding it is to measure the CO_2 tension of some gas from the last part of expiration. This, in most cases, is very close to Pa_{CO_2}. The CO_2 tension is derived, of course, from its concentration by the equation:

$$PA_{CO_2} = FA_{CO_2}\,(PB - 47)$$

where FA_{CO_2} is the percentage of CO_2 in the alveolar air expressed as a fraction, and PB is the barometric pressure. The alveolar CO_2 concentration FA_{CO_2} can be obtained by spot analysis or continuous analysis of end-expired air. Spot analysis is done by one of the chemical absorption methods such as Haldane's. A simpler absorption apparatus has been produced by Dräger in Germany. It can be used at the bedside and is sufficiently accurate for clinical purposes. It deserves to be better known.

There are at present two physical methods available for the measurement of FA_{CO_2}: infra-red gas analysis and sonic gas analysis. The principles on which they work have been described previously (Woolmer, 1953). Both are rapid methods, which can respond to the changes in FA_{CO_2} in a single breath. A whole breath may therefore be sampled rather than the last fraction of it.

There are two models of the infra-red gas analyser generally available in Britain; and one in the USA. A sonic gas analyser for CO_2 has been developed by Stott (personal communication, 1957), but is not yet generally available.

A physico-chemical method, which is continuous but not quick enough for single breath analysis, is that of Brinkman (Brinkman & Lamberts, 1952). It depends on the solution of CO_2 to form H_2CO_3 and the effect of this acid on an indicator. The colour change, which is proportional to FA_{CO_2}, is measured photoelectrically. Its clinical use has been described (Engell & Ibsen, 1953; Woolmer, 1956).

There are thus several ways of assessing the adequacy of ventilation, though unfortunately none of them is as simple and straightforward as could be desired. The principle underlying the management of respiratory inadequacy is simply the re-establishment of a ventilation sufficient to maintain Pa_{CO_2} within the limits of 35–45 mm. Hg. How this should be done depends upon the cause.

3. Poliomyelitis

In the paralytic form of the disease, involvement of anterior horn cells in the upper thoracic and lower cervical regions of the cord necessarily impairs the ventilation. It will produce ventilatory inadequacy at rest, however, only if the impairment is greater than can be compensated for by the patient's ventilatory reserve. This reserve is normally very large: the maximum breathing capacity is some twenty times the ventilation required at rest. It is, however, quite common for so many motor neurones to be put out of action by spinal poliomyelitis that the maximum tidal volume remaining to the patient is barely more than enough to wash out his dead space, so that gross under-ventilation results.

In spite of its many disadvantages, the cabinet respirator provides the best means of helping a patient with severe respiratory inadequacy from spinal poliomyelitis. Other mechanical devices such as the cuirass respirator (in one of its many forms), the rocking-bed or the rocking-chair, may be sufficient when the inadequacy is not severe. When respiratory inadequacy results from bulbar poliomyelitis, it is the central governor which is most affected. The receptor and effector mechanisms need not be involved. The bulbar form of the disease, however, is the most lethal, largely because its dangers are not universally understood. The routes by which air passes to the lungs and nourishment passes to the stomach cross over in the hypopharynx. Everyone knows that level-crossings can be dangerous. The crossing-gates are worked with extraordinary efficiency in health, and accidents are almost unknown.[1] The situation is quite different in conditions affecting the central control mechanism, when the signalman attending the crossing is struck down. Then, it is possible for traffic which should be confined to the oesophagus to enter the trachea. This, of course, may lead to respiratory inadequacy from derangement of the effector mechanism, either by producing obstruction or by inducing gross changes in the lungs. If poliomyelitis strikes, not only at the bulb but also at the motor nuclei in the upper part of the trunk, the helpless patient is particularly at the mercy of this threat, for he cannot prevent access of foreign matter into his trachea either by closing his glottis or by turning on to his face and so calling gravity to his aid.

The management of a patient with bulbar poliomyelitis, therefore, differs from that of one in whom the disease is confined to the spinal cord. If a patient who has lost control of his larynx, and who has no longer the ability to cough, is placed supine in a cabinet respirator, the powerful bellows action imposed on the chest by the respirator will draw into the undefended lungs any foreign matter, such as regurgitated stomach contents or secretions from the upper respiratory tract, lying in the hypopharynx. Hence respiratory inadequacy in the presence of bulbar paralysis must be corrected by means other than a cabinet respirator. It is now generally agreed that the best method is intermittent positive-pressure respiration through a tracheostome (Smith, Spalding & Russell, 1954).

There is no space, in this article, for a detailed consideration of the management of patients with bulbar palsy. Much has been learned about this in recent years, and the reader is referred to the monograph by Russell (1956).

4. Tetanus

Though at first sight it might seem that tetanus and bulbospinal poliomyelitis have little in common, there are, in fact, important similarities between them. Patients who die, from

[1] A simple demonstration of this can be obtained by the reader, if he will attempt to continue an inspiration while he is swallowing.

either disease, do so because of respiratory inadequacy and toxaemia. In each, the respiratory inadequacy results from a breakdown both in the central control mechanism and in the neuro-effector link. Hence the two diseases require similar management in some respects. Although it has been asserted that no treatment is of any avail if the amount of tetanus toxin absorbed is large (Christensen & Thurber, 1957), it is nevertheless possible, by proper treatment, to save the lives of many patients. A reduction of mortality is especially worth striving for in this disease, which affects the young and the strong; for recovery, when it occurs, is usually complete with no residual lesions.

Treatment consists in " neutralizing the uncombined toxin before it can produce further effects, eliminating the focus of infection and supporting the patient through this self-limited disease " (Christensen & Thurber, 1957). The best way of supporting the patient is to maintain his ventilation, to protect his lungs from invasion, and to prevent the anoxic crises which are so damaging. These anoxic crises occur during the spasms and convulsions which are the dominating features of severe tetanus. Until recent years, the only way to control these convulsions was by giving centrally acting depressants—such as opiates, barbiturates and paraldehyde, and even chloroform, in large doses; and this method has many successes to its credit (Cooke, 1953; Forbes & Auld, 1955; Adriani, personal communication, 1957). It has always seemed to me, however, that it is wrong in principle to give drugs which have a depressant effect on the central nervous system to a patient whose mid-brain is under attack both from tetanus toxin and from anoxia. A more logical alternative is to reduce the muscular hypertonia by drugs which act more peripherally in the nervous system. Of such drugs there are two groups available. Those in the first group act at the neuromuscular junction, either by competition or by depolarization; those in the second group are thought to act mainly on polysynaptic relays in the spinal cord. D-Tubocurarine chloride (Adriani & Ochsner, 1947; Frank, 1951; Segar, Littlefield & Walcher, 1952; Bjørneboe, Ibsen & Johnsen, 1954; Honey, Dwyer, Smith & Spalding, 1954), gallamine (Parkes, 1954), decamethonium (Keir, 1950) and succinylcholine (Woolmer & Cates, 1952; Forrester, 1954; Shackleton, 1954; Brinton & Burk, 1955) have been most used in the first group; and mephenesin (Belfrage, 1947; Davison, Ward & Pask, 1949; Nilsson, 1950; Godman, 1951; Plewes & Wilson, 1951; Veronesi & Meira, 1951) and zoxazolamine (Amols, 1956; Adriani, personal communication, 1957) in the second. The reason for using muscle relaxants in tetanus is this. Tetanus, like bulbo-spinal poliomyelitis, kills by respiratory inadequacy. The muscle spasms, the incoordination, the simultaneous contraction of agonists and antagonists and the disorganization of the larynx all contribute to this effect. Tetanus differs from bulbo-spinal poliomyelitis in that muscular rigidity is present instead of flaccidity, and that, because of hypertonia, anoxia crises occur. If, therefore, flaccidity can be substituted for hypertonia, these differences will be abolished. In the last five years the management of bulbo-spinal poliomyelitis has undergone a revolution which was initiated by the work of Lassen (1953) in the epidemic in Copenhagen; and as a result of new methods of treatment the mortality from this condition, which was very high, has been enormously reduced. It is only the hypertonia which makes this treatment difficult to apply in tetanus. Therefore, if the hypertonia is abolished, the patient with tetanus will have as good a chance of recovery as the patient with bulbo-spinal poliomyelitis, and, of course, without the likelihood of residual paralysis.

The management of a patient with severe tetanus according to these considerations is not easy, and it is even more exacting than is that of a patient with bulbo-spinal poliomyelitis (Woolmer & Cates, 1952). In a patient with tetanus, the degree of hypertonia varies from minute to minute, and the amount of relaxant required to neutralize it will vary correspondingly. To meet these variations and so to maintain *normal* tone, a short acting muscle relaxant, such as succinylcholine, is required, and its nice administration is often a matter of considerable difficulty. There is often a narrow margin between spasm and paralysis (Turner & Galloway, 1949), and some workers hold the view that the restoration and maintenance of normal tone is impracticable, and that it is better to paralyse the muscles and keep them flaccid. This, of course, involves the necessity of artificial respiration for long periods; but the supporters of the method accept this, and argue that failure to maintain normal tone—which is admittedly difficult —will in any case necessitate it. If continued flaccidity is aimed at, then a long acting muscle relaxant should be used.

The use of the second group of drugs has, perhaps, a still greater logical appeal. It has been shown that the tetanus toxin preferentially attacks those interneurones of polysynaptic reflex arcs which produce inhibition (Brooks, Curtis & Eccles, 1955). As a result, reciprocal inhibition of the antagonist is blocked, and agonist and antagonist both contract simultaneously. Mephenesin and zoxazolamine are both thought to act on those interneurones in the spinal cord that subserve excitation (Berger & Bradley, 1947; Amols, 1956). If this is true, they are proper pharmacological antagonists to the toxin of tetanus. My own experience with mephenesin has been less happy than that of some of its supporters. The solution may cause thrombosis if given intravenously, and oral medication, to be effective, requires many grams of the drug daily. The alcohol used as a vehicle may cause intoxication and throw an additional strain on an already intoxicated system. Zoxazolamine is less well known, but it seems to have certain advantages over mephenesin.

There have been several reports of the use of chlorpromazine as an adjunct to other drugs in the treatment of tetanus (Adriani & Kerr, 1955; Bodman, Morton & Thomas, 1955; Kelly & Laurence, 1956). The consensus of opinion corresponds with my own that this drug may have a remarkable effect in relieving spasm and stiffness in certain cases, but its pharmacological effects are so various that it is difficult to evaluate.

Whatever method is used for the control of the hypertonia, everyone with experience in this field agrees that tracheostomy should be performed early in the treatment of severe tetanus (Creech, Woodhall & Ochsner, 1950; Herzon, Killian & Pearlman, 1951; Forbes & Auld, 1955). In severe tetanus, as in bulbar poliomyelitis, the larynx can no longer fulfil its function as " the signalman at the level-crossing ", and it is safer to re-route the airstream. Re-routing alone, however, is not enough. One has also to make sure, for complete safety, that all other traffic is barred from the route allotted to the airway. One way of doing this is the insertion of a tube with an inflatable cuff through the tracheostomy into the trachea. Another way is the establishment of a gastrostomy which permits the abandonment of oral feeding.

The treatment of severe tetanus and of bulbo-spinal polio-

myelitis is difficult and exacting. The maxim that prevention is better than cure is especially applicable to these two diseases. Happily, the increasing use of antitetanus toxoid, and of antipoliomyelitis vaccine, is already lessening their occurrence.

5. Narcotic Poisoning

No such decrease is discernible in the incidence of respiratory inadequacy from narcotic poisoning, from carbon-monoxide intoxication, or from head injuries; and the principles governing the treatment of this condition should be widely understood. They are, simply, the establishment and maintenance of a free airway, the provision of adequate respiratory exchange, the protection of the lungs from invasion and the countering of severe circulatory depression. Secondary considerations, such as the maintenance of fluid balance and nutrition, and proper nursing, cannot be dealt with here. The use of cerebral stimulants, such as picrotoxin, has been recommended, in earlier years, to cut short the period of unconsciousness. However, there is a danger that the use of cerebral stimulants may increase the demand for oxygen of the brain-cells so that it outstrips the supply, and this will result in more harm than good. Unconsciousness is not in itself dangerous, provided that the patient can be given proper attention, and better results have been obtained (Nilsson, 1951; Clemmesen, 1954; Eckenhoff & Dam, 1956) from a regime based on attention to first principles and good nursing. The introduction of such drugs as β-ethyl-β-methyl-glutarimide (Megimide), which were thought to have a specific antagonism to the barbiturates rather than a general stimulant effect (Shaw, Simon, Cass & Shulman, 1954), and 2 : 4-diamino-5-phenylthiazole (Daptazole), which appears similarly to antagonize the opiates (Shaw & Shulman, 1955), calls for a re-examination of the pharmacological treatment of narcotic poisoning. The evidence about the usefulness of these drugs in the treatment of narcotic poisoning, or about the specificity of their action, is at present inconclusive (Shulman, Shaw, Cass & Whyte, 1955; Clemmesen, 1956; Kjaer-Larsen, 1956; Pedersen, 1956; Boyan, Howland & Bellville, 1957; Plum & Swanson, 1957). They do not lessen the concentration of narcotics in the blood, and they seldom shorten coma, but they can lessen respiratory depression. It would seem that they may have some use as adjuvants, but that their introduction in no way affects the principles of treatment, which remain as indicated.

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ELECTROGRAPHIC MONITORING OF ANAESTHESIA

Application of Electroencephalography to Surgical Anaesthesia

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1. Principles of Electrographic Monitoring of Anaesthesia

The value of the application of the principles of clinical electroencephalography[49] to anaesthesia is now becoming apparent to anaesthetists[67 27 81 25 58 79 62 102 104 47].[a] This arises from the fact[53 49 50 100] that the dimensions of the electrical activity of the brain reflect the state of metabolism of the neurones of which it is comprised. Analysis of records of this activity during an operation therefore reveals to the anaesthetist not only the extent to which cerebral metabolism is specifically modified by the anaesthetic agent, but also the degree to which this pharmacological action is being influenced by the attendant variations in the circulatory, respiratory and general biochemical functions of the individual that inevitably arise[89 57 94 56] during surgical procedures.

Ideally, anaesthesia should be a process of controlled, reversible intoxication of the nervous system. In these terms it may then be regarded as a dynamic equilibrium process extended in time, the level of which may be altered either by changes in the fraction of drug in the blood available to effect narcosis (the pharmacologically effective fraction[103]), or by changes in the narcotic responsiveness[103] of the brain—which latter depends on the level of cerebral metabolism. It thus follows that the electrographic patterns displayed throughout an operation by the brain of an anaesthetized patient are a measure of the resultant cerebral activity produced by the interaction of these two variables. Sequential analysis by the anaesthetist of his patient's brain-potential patterns then provides a practical method for continuous quantitative assessment of that patient's response to administration of an anaesthetic agent or agents under all circumstances.

The essence of the anaesthetist's ability to control the deliberate distortion of neural metabolism which he induces in his patients lies in the skill with which he can evaluate its course. This he must do in the light of the influence, not only of the anaesthetic agent or agents, but also of a multiplicity of attendant factors which inevitably arise during surgical procedures. Until now this skill has been developed only by long and arduous practice, by dint of which the anaesthetist eventually acquires a considerable body of empirical lore—the "clinical signs of anaesthesia"—upon which he then relies for assessment of the progress of narcosis[48 33 63 29].

But the multiplying variety of pharmacological agents now at the anaesthetist's disposal, coupled with the increasing complexity of surgical procedures in which he is ever more intimately involved, places an increasing strain on this empiricism. For the same reasons, it has become impossible to codify the "signs of anaesthesia" for every set of circumstances now likely to be encountered in anaesthetic practice. In this situation, therefore, scientifically-minded anaesthetists are seeking means whereby the semi-intuitive assessment of the progress of an anaesthetic procedure, which has long been the basis of their professional art, might be supplemented by quantitative methods. Such a demand for continuous measurement of the degree of anaesthetic intoxication of the central nervous system under the conditions obtaining during surgical operations can now be met by analysis of a continuous record of the extent to which the electrical activity of the patient's brain is being modified by the anaesthetic agent or agents.

This application of electroencephalography to surgical anaesthesia—referred to as "electrographic monitoring of anaesthesia"[105 102 103 104]—may be considered from two principal aspects. First, the technique may be employed by the anaesthetist to measure precisely, and continuously throughout an operation, the depth of anaesthesia, thus guiding himself in his administration of the anaesthetic agent or agents. Second, analysis of the tracings will reveal changes in cerebral metabolism associated with alterations in cerebral blood flow, in the gaseous and electrolyte composition of the blood, and in its glucose concentration—all of which influence the response of the brain to narcotics delivered to it. In this way, the patient's electroencephalogram is a sensitive index of the development of a number of complications of anaesthesia, revealing—at an early stage—the need for supportive measures to combat them. However, limitations of space permit discussion of only the first of these aspects, and material relevant to the second will be found amongst the references cited at the end of this article [64 45 18 88 75 44 73 50 101 43 25 54 70 65 68].

2. Technique of Electrographic Monitoring of Anaesthesia

The general principles and basic techniques of clinical electroencephalography[49] need but little modification in the operating theatre for control of anaesthesia, and hence are not redescribed in detail here.

[a] It may be of significance in this respect that one of the first Registrar's Prizes in Anaesthesia was awarded last year to a dissertation on electroencephalography in anaesthesia (see Wilson, S. M. (1957) *Proc. R. Soc. Med.* 50, 105).

In electrographic monitoring of anaesthesia in man, recordings are made from electrodes fixed to the scalp before anaesthesia commences[99]. If only one amplifier channel is to be used, two electrodes are applied—one on the forehead and another on the occiput—with an earth electrode over one or other mastoid process. For more detailed studies larger numbers of electrodes may be used[99 104]; but for routine procedures a pattern of eight electrodes symmetrically distributed over the head will be found to be very satisfactory[104], if sufficient amplifier channels are available for bipolar recordings from them. If the standard electrode positions[99 104] be employed, their presence on the patient's head does not interfere with its manipulation for purposes of intubation or fixation in a head-rest.

Where multi-channel electroencephalographs are available to the anaesthetist, precordial electrodes may be attached to the chest for simultaneous electrocardiography; while additional channels may be linked to special devices for recording electrically at the same time arterial blood pressure, respiration, oxygen saturation of the blood, carbon-dioxide content of the expired air, or body-temperature. In neurosurgical procedures involving craniotomy, electrodes mounted in a special holder attached to one of the cut edges of the skull[66 100 72] may be used to monitor the activity of the exposed cerebral cortex. In special cases electrodes may also be inserted into the brain substance[17 71], to study the electrical activity of intracerebral structures during anaesthesia.

Immediate visual analysis of the resulting records, in terms of the frequency, amplitude and pattern of activity, is performed throughout the operation. Administration of the anaesthetic agent by the anaesthetist is then determined by his interpretation of changes in these characteristics of the record, in relation to the particular operative procedure. In addition, as mentioned above, the occurrence of specific alterations in the record will indicate to him—often before overt clinical signs are manifest—the onset of anoxia[64 45 75 44 50 43 54 70], carbon-dioxide retention[45 18 44 73 50 43 25 65 68], hypoglycaemia [45 88 50 101], surgical shock or other complications of anaesthesia.

Inspection of such records reveals obvious differences in the patterns of electrical activity that develop as anaesthesia waxes and wanes; and it is important to stress that this type of analysis detects fluctuations in anaesthetic level which are little if at all revealed by the gross reflex changes upon which the anaesthetist customarily depends. These electrical indications therefore forewarn him of alterations in the level of the pharmacologically effective fraction of the circulating drug[103], or of changes in the brain's response[103] to it, some considerable time before he is otherwise aware of them. Further, once the anaesthetist has familiarized himself with the sequence of changes in the pattern of cerebral electrical activity produced by the anaesthetic agent (or combination of agents) that he customarily employs, it becomes a simple matter for him to induce anaesthesia precisely to any predetermined level and to maintain that level for as long as may be desired, or to change to any other level.

3. Analysis of Electroencephalographic Changes Produced by Anaesthetic Agents

A detailed analysis of the sequence of changes in cerebral electrical activity associated with the various anaesthetic agents, and their related clinical features, is not presented here as these data are readily accessible in published reports.

Suffice it to say that such analyses are available with human material for all the commonly used anaesthetic agents, in many cases correlated with parallel assays of the circulating blood level of the anaesthetic agent. Thus electrographic monitoring is a technique now applicable to all types of general anaesthesia; and it has already been utilized clinically with gaseous agents such as chloroform[11], ether[67 27 34 25 2 6 21 47], nitrous oxide–oxygen[35 28 67 82 27 34 25 21 47], cyclopropane[77 74 7] and xenon[69], as well as with intravenous agents such as the barbiturates[20 38 19 97 98 104 59 80 31 17 72] and the steroid anaesthetics[26 9]. Similar data are also available for many of the drugs employed as ancillary agents in anaesthesia, such as morphine and its related compounds[90 95 96 31 37], atropine [90 37], cocaine and procaine[90], and the relaxants[46 78 23 85 24 91].

To illustrate the application of electrographic monitoring to gaseous anaesthesia, on the one hand, and to intravenous anaesthesia on the other, two analyses only are given briefly here—one for a nitrous-oxide–oxygen–ether mixture, the other for thiopentone.

a. Nitrous oxide–oxygen–ether[35 28 82 34 47]

If induction of anaesthesia be performed with a mixture of 80% nitrous oxide and 20% oxygen (no thiopentone being given), followed by administration of 50% nitrous oxide and 50% oxygen, with admixture of increasing concentrations of ether, a regular sequence of cerebral electrical changes is observed. For clinical purposes this spectrum of changes in cerebral electrical activity[b] may be arbitrarily divided into four principal stages—but it should be noted that such stages, as determined electrically, do not necessarily correspond with the traditional stages of clinical anaesthesia.

Stage I. As induction develops, there is a rapid diminution in the voltage of all cerebral electrical activity, with desynchronization of normal α spindles into continuous, low-voltage fast activity at a variety of frequencies up to about 32 cyc./sec. These appearances correspond with the so-called "excitement" phase of induction, consciousness being lost.

Stage II. After about five to seven minutes the record changes abruptly as light surgical anaesthesia develops, there being a rapid build-up of rhythmically discharging, high-voltage δ waves at 2–3 cyc./sec. At first appearing intermittently out of the low-voltage background, these slow waves soon form an almost continuous, bilaterally synchronous discharge in which fast waves cannot be seen. At this stage the patient is unconscious and analgesic, but there is no muscular relaxation.

Stage III. With further deepening of anaesthesia this regular pattern of discharge becomes irregular in voltage and frequency, very slow high-voltage waves at 1–2 cyc./sec. being mixed irregularly with lower voltage waves at 2–4 cyc./sec. This is followed by gradual disappearance of the faster δ components and an over-all diminution in voltage of the record, as deep surgical anaesthesia develops. As a result, the pattern now consists of isolated or grouped waves at about 1–2 cyc./sec., separated by intervals of about 2–3 seconds during which little activity can be detected apart from slight unsteadiness of the base-line. The patient is now deeply unconscious, muscle reflexes are absent, and there is marked relaxation.

Stage IV. Should anaesthesia be deepened further, so that there is appreciable diminution of pulmonary ventilation, the amplitude of the isolated slow waves continues to diminish, while the inactive intervals between them double or treble in duration.

Recovery. When anaesthesia is discontinued, the above sequence is retraversed in the opposite direction as the blood concentrations of the anaesthetic gases diminish. However, it should be noted that, while the change from patterns indicative of deep anaesthesia to those associated with light anaes-

[b] Illustrations of tracings made during administration of this type of anaesthesia may be found in the references[35 28 82 34 47].

thesia is associated with the expected clinical changes, α activity may return at the lightest levels of anaesthesia before the patient regains consciousness—but the α rhythm does not return to its pre-anaesthetic stability until he does so. Further, it must be pointed out that the above sequence of events occurs only in adolescents and adults; in young children the patterns are somewhat different, as in such subjects the adult pattern of cerebral electrical activity has not yet been achieved.

b. Thiopentone[38 19 97 98 104 59 80 31]

The sequence of changes induced in cerebral electrical activity by barbiturate narcosis is similar for all drugs of this type. The only difference between individual drugs lies in the time course of the changes—particularly those associated with light levels of narcosis, during which the sequence is telescoped considerably with the short acting agents.

For electroencephalographic purposes the course of waxing human barbiturate narcosis may be arbitrarily divided into five stages[97 98 104], each of which is characterized by a particular pattern of cerebral electrical activity as well as by overt clinical phenomena[97], such as changes in reflexes and in respiratory and circulatory function. However, as noted above, with rapidly acting derivatives such as thiopentone and thialbarbitone the electrical patterns characteristic of the earlier stages of narcosis are merged to various degrees, unless the drug be given in dilutions greater than those customarily employed in anaesthetic practice.

With slow induction of thiopentone anaesthesia, using 10–12 mg. of drug/kg. body-weight, the following changes[e] may be observed.

Stage I. First there is rapidly developing instability of the resting rhythm, together with an increase in the voltage of the fronto-temporal β (16–32 cyc./sec.) activity. Occipital α spindles diminish in duration, and low-voltage β activity appears superimposed upon them. Slower frequencies steadily develop in the α band, and their voltage fluctuates more and more.

Stage II. Then the fronto-temporal β activity commences to spindle at about 16 cyc./sec., these spindles periodically interrupting the random faster β activity, while the slowing and irregularity of the occipital α activity become still more marked. The patient is now drowsy and often yawning, but is readily roused by gentle stimulation—although he is then slightly confused. Respiration commences to slow, and some peripheral vasodilatation is generally apparent by this time.

Stage III. The next stage is indicated by complete disappearance of occipital α spindles, and randomization of slow α frequencies at 7–9 cyc./sec. At the same time the faster β components disappear from the fronto-temporal regions, leaving prominent spindles at 14–16 cyc./sec. These spindles rapidly increase in voltage; and at the same time slower θ waves emerge in the fronto-temporal areas and spread into other regions of the brain. This activity, at 4–6 cyc./sec., appears first as random discharges; but, as anaesthesia deepens, prominent θ spindles lasting 1–2 sec. develop. The patient is now lightly anaesthetized, with evident slowing and deepening of respiration, definite cutaneous vasodilatation and commencing muscular relaxation. Stimulation still rouses him incompletely, however.

Stage IV. Spindling at 14 cyc./sec. now increases markedly in the fronto-temporal areas, and spreads to other regions. At the same time the random and spindled θ activity becomes wide spread, even in the occipital leads, where it soon submerges the remaining α frequencies; and it contains an increasing proportion of frequencies at 4 cyc./sec. The patient is now in light surgical anaesthesia, with moderate muscular relaxation and diminished reflexes; but painful stimulation may partly rouse him, and diminish the muscular relaxation.

Stage V. The onset of deeper surgical anaesthesia is indicated by a reduction in voltage of all activity. In the fronto-temporal areas this is especially conspicuous with the β spindling, which is now replaced by low-voltage discharges at 6 cyc./sec. In other areas of the brain the faster frequencies disappear, and there is diffuse random activity at 3–4 cyc./sec., with little spindling. This latter activity continues to slow through the δ band, while frequencies faster than 3 cyc./sec. gradually disappear everywhere. Stimulation now will not rouse the patient, and muscular relaxation is marked, the reflexes being absent. Should anaesthesia deepen still further, the appearances come to resemble those described for deep gaseous anaesthesia, in that in all areas of the brain there are intermittent low-voltage discharges at 1–2 cyc./sec. occurring in isolation or in small bursts, separated by intervals of several seconds during which little activity is detectable.

Recovery. Recovery from anaesthesia is accompanied by the reverse sequence of events. The patterns characteristic of the deeper levels of anaesthesia are retraversed rapidly; but, as lighter levels are reached, the rate of change diminishes steadily. The patient becomes increasingly responsive to stimulation as 14 cyc./sec. spindles become prominent in the fronto-temporal regions, and α activity returns in the occipital areas; but even after the patient regains consciousness the α activity remains unstable, and there is more fast β activity than in the pre-anaesthetic record, perhaps for an hour or more. While this picture obtains, the patient readily falls off to sleep, if he is not in pain and is left undisturbed. Finally, it must again be pointed out that this sequence of events is observed only after cerebral electrical activity attains maturity; the appearances during thiopentone anaesthesia in infants and young children [4] differ considerably.

[e] Representative tracings are depicted in the references[38 19 97 98 104 59 80 31].

4. Applications of Electrographic Monitoring of Anaesthesia

a. Control of Anaesthesia

Using this sort of information, it will be apparent that the anaesthetist, having decided upon a particular anaesthetic agent or combination of agents, can select any particular level of anaesthesia (as determined by the pattern of cerebral electrical activity) by administering the anaesthetic until the desired pattern is achieved. He can then maintain this level precisely for as long as desired, by giving more of the drug or gas (in appropriate amounts and concentrations) as and when indicated by commencing deviation of the appearances of the record from the pre-selected pattern. Should he wish to change to a deeper or lighter level of anaesthesia during the course of the operation, he may give either more or less of the drug or gas until the desired pattern is achieved—at which point the narcotic equilibrium can be re-established. Finally, at the termination of the operation the anaesthetist can measure the adequacy and rate of the patient's recovery from anaesthetic intoxication, by observing the devolution of the succession of patterns characteristic of the various levels of anaesthesia in a record which continues after he ceases to administer the anaesthetic.

It may not be unreasonable then to suggest that the phenomena of electrographic monitoring of anaesthesia constitute a practically useful and reliable measure of the progress of anaesthetic intoxication of the central nervous system for the anaesthetist who deliberately induces it, and provide a means of supplementing quantitatively the qualitative observations which he has learned to make during his surgical experience.

b. Teaching of Anaesthesia

Further, it would seem that some of the difficulties experienced in teaching anaesthesia by empirical methods, and the length of time at present required to achieve competence in the art, might be reduced by employing electrographic monitoring as a teaching aid. For, if the student can be shown that the devolution of the "clinical signs of anaesthesia" is accom-

panied by precise changes in the amplitude and frequency of the brain waves which he can be taught to recognize and measure, and that both sets of phenomena depend upon related neurophysiological mechanisms[81 83 84 43 42 40 12 1 31 32 76 39 61 60 103], he may perhaps realize that he is acquiring a science as well as an art, and thus feel his feet to be on firmer ground.

Nevertheless, he has to take the trouble to learn to interpret the records, and this can be done only in practice. Therefore although electrographic monitoring may be a scientific aid to the practice of anaesthesia, it cannot replace the skill acquired by long experience of anaesthesia in the operating theatre—it can only supplement and refine that skill.

c. Servo-Anaesthesia

In spite of this, attempts have been made to simplify the anaesthetic procedure still further, by eliminating the anaesthetist from the scene; for the observations described above have led to the development of techniques by which analysis of cerebral electrical activity during surgical anaesthesia may be made automatically, rather than by the anaesthetist's visually inspecting the records of his patient's brain waves. In this system of so-called "servo-anaesthesia"[13 14 15 16 92 86 60 7 30], analysis of the electrical activity of the patient's brain is performed continuously by an electronic integrator which in turn controls, through relaying devices, the rate of administration of the anaesthetic agent (whether drug or gas mixture).

The principle of automatic analysis of human cerebral electrical activity is now familiar to clinical electroencephalographers, largely as a result of its development and application by Grey Walter and his colleagues[93]. As applied to the control of anaesthesia, the technique consists in integration of the voltage output of the brain within the frequency limits of the electroencephalographic amplifiers, this voltage output being considered as a function of the energy production of the brain. The integration is performed electronically, by an apparatus which rectifies the potential signals from the electroencephalographic amplifiers. The output from the integrator is then delivered through a relay system to a motor, which either drives the piston of a syringe containing thiopentone solution or else, through appropriate devices, regulates the valve on a cylinder of anaesthetic gas.

Should the voltage output from the brain increase (indicating waning of narcosis), then this system will accelerate the rate of delivery of the anaesthetic agent to the patient; while if the rate diminishes, the rate of supply of drug or gas will be correspondingly reduced. In short, this system consists essentially in automatic variation in the rate of flow of a continuously administered anaesthetic solution or gas in response to variation in certain aspects of cerebral electrical activity.

The technique of servo-anaesthesia is thus fundamentally different from the technique of electrographic monitoring, in that the latter generally involves intermittent administration[d] of the anaesthetic agent by the anaesthetist, who then functions as both "integrator" and "servo-motor" in the anaesthetic circuit. In the present state of affairs it will still be found more satisfactory to employ the trained neuronal circuits of the anaesthetist in the system in preference to an automatic integrator, for experience with the technique of servo-anaesthesia in the laboratory and operating theatre has revealed several practical difficulties—difficulties which the originators of the method have not failed to point out[16 92 60]. One of these is the fact that the integrator, unlike the trained operator, cannot discriminate between signals coming in actual fact from the patient's brain and adventitious signals derived from electrostatic sources, a.c. interference, and movements of the patient or the operating team. All input signals are grist to the integrator's mill, seeming to indicate an increased energy output from the brain which demands more anaesthetic. This difficulty might appear to be resolved by inserting, as the workers at the Mayo Clinic have done[16], a narrow band-pass frequency filter on the input side of the integrator. But there are still practical difficulties in such systems, particularly if the anaesthetist wishes to work at a variety of levels of anaesthesia at different stages of an operation[92], although they cannot be considered further here.

Automatic control of anaesthesia may be in itself a most desirable end, in that it may free the anaesthetist from the mechanical drudgery of administering a gas or drug for more vital tasks concerned with the patient's welfare, during complex operative procedures. But as things stand at present, it is clear that intermittent regulation of the administration of the anaesthetic agent by the anaesthetist in the light of his visual analysis of a monitoring brain-potential record and of his experience of operative procedures is a simple and immediately available technique, contributing to the safety of his patient, the efficiency of his anaesthetic procedure, and the equanimity of his surgical colleagues.

5. Some Further Considerations

There are many other aspects of electrographic monitoring of anaesthesia that are of practical significance, but they cannot be dealt with in detail here. However, a few of them may be mentioned briefly in order to indicate something of the scope of the subject.

a. Light Anaesthesia with Relaxants

One of the problems inherent in the current practice of performing operations under very light anaesthesia[3] while securing muscular relaxation by administration of neuromuscular blocking agents, is the difficulty of determining the "level" of anaesthesia from traditional clinical signs, for many of them are absent in these circumstances. If the anaesthetist's object be to maintain a steady level of anaesthesia that is light, and yet deep enough to ensure that the patient is unaware of what is going on and is amnesic for the procedure after it is completed, electrographic monitoring of anaesthesia is a technique at hand with which he can achieve this aim with certainty. It will also provide him with a sensitive index of the efficiency with which he is controlling the patient's pulmonary ventilation, in view of the changes produced in the electroencephalogram by anoxia and carbon-dioxide retention (see above, section 2).

However, it should be noted in passing that most of the relaxants—including curare and its derivatives, and gallamine triethiodide—have potent influences on the central nervous system in addition to their more obvious peripheral action. For this reason they themselves produce characteristic changes in the electroencephalogram[46 78 23 85 24] which are superimposed upon—and must be distinguished from—the effects produced by the primary anaesthetic agents in interpreting a monitoring record.

[d] Except where variations are made in the flow-rate of a continuously supplied gas mixture.

b. Induced Hypothermia

Because the spectrum of cerebral electrical activity reflects the metabolic status of the neurones comprising the brain—and especially those in the brain-stem[1 104]—it follows that, as this metabolic activity is altered by cooling the brain, sequential changes in the electroencephalogram will accompany reduction in body-temperature. Many studies have shown that electroencephalographic activity is directly related to temperature changes in the brain[52 53 22 55 41 10 106]; and therefore electrographic monitoring of anaesthesia is a useful guide to the extent to which cerebral metabolism is reduced by hypothermic procedures[36 51].

Further, as these metabolic changes will alter the narcotic responsiveness of the brain[103], it follows that the patterns of electrical activity characteristic of particular levels of anaesthesia will be profoundly modified as the patient is cooled; and account must be taken of this in employing electrographic monitoring to control the depth of anaesthesia in the hypothermic patient. In spite of this apparent complication, however, the technique will be found to be of great help in maintaining the light levels of anaesthesia needed for operations during induced hypothermia.

c. Induced Hypotension

As cerebral metabolism—and particularly the metabolism of the highly vascular basal regions of the brain—is dependent on the blood flow through the brain, it is clear that cerebral electrical activity will be greatly modified should that blood flow be diminished[5 87], as when the blood pressure is artificially reduced by use of ganglion-blocking agents. Such changes in cerebral blood flow are reflected in marked slowing of cerebral electrical activity, even at very light levels of anaesthesia. In these circumstances then, the considerations as regards interpretation of the tracings are similar to those affecting induced hypothermia; and electrographic monitoring may be employed as a means of controlling the induction and maintenance of hypotension, because of its extreme sensitivity as a method of determining the adequacy of the cerebral blood flow.

d. Extra-Corporeal Circulation

For the reasons mentioned in the previous paragraph, together with the fact that the electroencephalogram is extremely sensitive to changes in the oxygen saturation of cerebral arterial blood as well as to the magnitude of that flow, it should be clear that electrographic monitoring is a useful procedure in operations carried out with an extra-corporeal circulation[70 8]. By careful attention to his patient's electroencephalogram in such circumstances, the anaesthetist can readily and directly satisfy himself that the apparatus maintaining the artificial circulation is providing a sufficient flow of adequately oxygenated blood to his patient's brain throughout the whole period of its use. Should the apparatus fail to do so, the fact will be readily appreciated from inspection of the monitoring electroencephalogram some considerable time before the anaesthetist may otherwise be aware of it.

6. Conclusion

Conventional criteria for assessing anaesthetic depth, such as observation of changes in muscle reflexes or in respiratory pattern, are hardly precise enough for many of the purposes of modern anaesthesia. However, studies such as those referred to here have now clearly established that sequential changes may be observed in brain-potential patterns during narcosis with various agents, both in animals and in man; and this information may be utilized in practice for control of the level of anaesthesia.

Detailed analysis of the cyclical pattern of cerebral electrical response that develops during waxing and waning anaesthesia, two examples of which are presented here, has shown that various arbitrary stages may be identified in the devolution of brain-potential changes produced by all anaesthetic agents. These empirically selected brain-potential patterns may be correlated with changes in a variety of somatic and autonomic reflexes, and their study permits identification of various levels of narcosis.

Continuous inspection of the cerebral electrogram during an operation is then a simple method of recognizing and controlling fluctuations in depth of anaesthesia—fluctuations often not revealed by more conventional methods. In this way the anaesthetized subject may be kept at a steady level of narcosis by methods which maintain an unvarying pattern of cerebral electrical activity: for selected levels of anaesthesia may be reached and maintained by controlling the administration of the anaesthetic agent (or agents) in the light of visual inspection of a monitoring brain-potential record. Even in complex operative procedures this method provides a continuous and precise control over anaesthesia that is more efficient than is the case with conventional empirical methods, and with techniques rather more simple than those involving servo-anaesthetic systems.

It is therefore suggested that, while automatic integration methods may ultimately prove to be even more efficient, visual analysis of a monitoring record provides at present a simple and readily available method for routine use in the operating theatre, by which constant surveillance may be maintained over the welfare of any anaesthetized patient.

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THEORETICAL CONSIDERATIONS OF THE EFFECTS OF REBREATHING IN TWO SEMI-CLOSED ANAESTHETIC SYSTEMS

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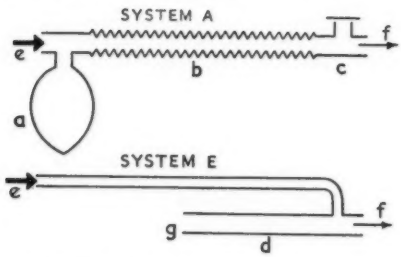
1 System A (" Magill's attachment ")
2 System E (" T-piece " arrangement)
Appendix
References

In a previous paper (Mapleson, 1954) the minimum flow-rate of fresh gases needed to eliminate rebreathing in five semi-closed anaesthetic systems was determined theoretically. Many of these determinations have since been confirmed by experimental work in the laboratory (Woolmer & Lind, 1954; Bracken & Sanderson, 1955; Inkster, 1956) and in the operating theatre (de Clive Lowe, 1956; Davies, Verner & Bracken, 1956; Elam & Brown, 1956). In the present paper it is proposed to extend the analysis to cases where rebreathing does occur.

Of the five systems considered in the earlier paper, system A (fig. 1), often referred to as " Magill's attachment ", had the unique advantage that rebreathing could be eliminated by a fresh gas flow which was about equal to the patient's minute volume respiration. System E (fig. 1), often referred to as the " T-piece " arrangement, had the unique advantage of dispensing with an expiratory valve, and hence its accompanying resistance, but the disadvantage of requiring a fresh gas flow of two or more times the patient's minute volume to eliminate rebreathing. The remaining systems combined the disadvantages of systems A and E in that they all involved expiratory valves and all required fresh gas flows of at least twice the minute volume. They will therefore not be considered here.

FIG. 1. DIAGRAM OF ANAESTHETIC SYSTEMS



a: reservoir bag
b: corrugated tubing
c: expiratory valve
d: limb of T-piece
e: constant gas flow from anaesthetic machine
f: to face-mask (or endotracheal tube) and patient
g: open to atmosphere

1. System A (" Magill's Attachment ")

In this analysis, as in the previous paper, it will be assumed that:

i. The system is in a stable state—that is, that there is anaesthetic equilibrium and that successive respiratory cycles are identical.
ii. The expiratory valve opens at some critical pressure, slightly above atmospheric, and passes large flows for no extra drop in pressure.
iii. The expiratory valve closes immediately at the start of inspiration. This implies that at the start of inspiration the inspiratory flow-rate rises instantaneously to some value greater than the steady flow of fresh gas from the anaesthetic machine. No other assumption need be made as to the nature of the respiratory pattern.
iv. No longitudinal mixing occurs in the corrugated tube or the patient's dead space.

The first assumption merely restricts the analysis to steady conditions. In the previous paper it was shown that errors due to the second and third assumptions were small. The fourth assumption serves to keep the analysis simple but does introduce appreciable errors which will be discussed later.

During a complete cycle a volume, $\dot{V}F/f$, of fresh gas enters the system from the anaesthetic machine, where $\dot{V}F$ is the fresh gas flow-rate and f the frequency of respiration. In the steady state, an equal volume must escape from the system in each cycle. In the previous paper it was shown that, with no longitudinal mixing, no fresh gas leaves the system through the expiratory valve unless the whole of the patient's expiration also does so. Therefore, in the presence of rebreathing, the volume of gas, $VESC$, which escapes from the system in each respiration is equal to $\dot{V}F/f$ and consists entirely of expired gas. Let the volumes of gas expired and inspired in each cycle both be equal to VT. (The error involved in this simplification is negligible compared to that due to assuming no longitudinal mixing.) Then a volume, $VT - VESC$, of expired gas must remain in the system at the end of expiration. But it was also shown that the portion remaining in the system comes from the first part of expiration. It therefore contains a volume, VD (the volume of the dead space), which has not been in contact with the alveoli and so is still fresh gas. The remainder has come from the alveoli and so is alveolar gas (remembering the assumption of no longitudinal mixing) and has a volume, $VT - VD - VESC$. This is the alveolar gas which is rebreathed on inspiration. However, a further volume, VD, of alveolar gas is inspired from the dead space. Therefore the total volume of alveolar gas drawn into the alveoli on inspiration is

$$VT - VD - VESC + VD = VT - VESC$$

Let the concentration of CO_2 in alveolar gas be FA_{CO_2} (expressed as a fraction and not as a percentage). Then, during inspiration, a volume, $FA_{CO_2}(VT - VESC)$, of CO_2 enters the alveoli. Also, let the CO_2 output of the patient be $\dot{V}CO_2$ so that the CO_2 output per respiration is $\dot{V}CO_2/f$. Then the volume of CO_2 leaving the alveoli during the next expiration (in the steady state when there is no accumulation of CO_2) must be $FA_{CO_2}(VT - VESC) + \dot{V}CO_2/f$. But the total volume of gas

leaving the alveoli during expiration is VT (see above) and this contains a fraction, FA_{CO_2}, of CO_2 and hence a volume of CO_2 equal to $FA_{CO_2}VT$. Thus

$$FA_{CO_2}VT = FA_{CO_2}(VT - VESC) + \dot{V}CO_2/f$$

Whence, remembering that $VESC = \dot{V}F/f$,

$$FA_{CO_2} = \frac{\dot{V}CO_2}{\dot{V}F} \qquad (1)$$

It should be noted that this result is obtained only in the presence of rebreathing. When $\dot{V}F > f(VT - VD)$ no alveolar gas is left in the system at the end of expiration and so rebreathing is eliminated. Then it can be shown that

$$FA_{CO_2} = \frac{\dot{V}CO_2}{f(VT - VD)} \qquad (2)$$

Thus, in both cases, the alveolar concentration of CO_2 is equal to the rate of CO_2 output from the lungs divided by the rate at which fresh gas is supplied to the alveoli. In the absence of rebreathing (2), the rate at which fresh gas reaches the alveoli is limited only by the patient's respiration and is equal to the effective minute volume, $f(VT - VD)$. When rebreathing occurs (1) the rate is limited by, and is equal to, the fresh gas flow-rate, $\dot{V}F$.

It should be remembered that the extremely simple result, expressed by (1), applies only to steady conditions. If $\dot{V}F$ or $\dot{V}CO_2$ are suddenly changed, the alveolar concentration of CO_2 will gradually change from its old level to one given by the new values.

It is important to note that, in these circumstances, according to (1), the alveolar CO_2 is independent of the respiration. Thus, with this system, the patient cannot compensate for a low gas flow-rate by breathing more deeply or more rapidly —though matters could be made worse by a sufficient reduction in minute volume. The physical explanation of this phenomenon is that if the patient increases, say, his tidal volume, then the larger volume of gas drawn into his lungs on inspiration contains a correspondingly larger volume of alveolar gas left in the system at the end of the previous expiration.

It should be stressed that these results depend on the absence of longitudinal mixing of gases in the corrugated tube and dead space. In a practical case, where mixing does occur, the complete elimination of rebreathing will require a somewhat higher fresh gas flow-rate than that defined by $\dot{V}F = f(VT - VD)$. In the previous paper the condition $\dot{V}F \geqslant fVT$ (fresh gas flow-rate equal to or greater than the minute volume) was suggested and has since been supported experimentally. Also, when $\dot{V}F \simeq f(VT - VD)$, the alveolar CO_2 will be somewhat higher than the value given by (1) or (2). Further, a limited amount of compensation by overbreathing will be possible but (1) represents the minimum proportion of CO_2 in the alveoli.

In the laboratory experiments of Woolmer & Lind (1954) on system A, the results were expressed in terms of the mean percentage of CO_2 inspired from the system. From the present analysis this can readily be shown to be

$$100\,\frac{\dot{V}CO_2}{\dot{V}F} \cdot \frac{VT - VD - \dot{V}F/f}{VT}$$

when rebreathing occurs. The values calculated according to this formula are compared with the averages of Woolmer and Lind's observed values in Table I. It is seen that the agreement is quite good although, as predicted above, discrepancies due to longitudinal mixing do show up with fresh gas flows close to that at which rebreathing is just eliminated according to the above theory: that is, at $\dot{V}F = 4$, 5.6 and 8.8 l./min. for $VT = 400$, 500 and 700 ml. respectively.

TABLE I. COMPARISON OF CALCULATED VALUES OF MEAN PERCENTAGE OF CARBON DIOXIDE INSPIRED IN SYSTEM A WITH THOSE OBSERVED BY WOOLMER & LIND (1954)

Fresh gas flow (l./min.)		10	7	5	3	2
Tidal volume = 400 ml.	Calculated	0	0	0	1.2	3.5
	Observed	0.12	0.12	0.22	1.4	3.4
Tidal volume = 500 ml.	Calculated	—	0	0.34	—	—
	Observed	—	0.16	0.44	—	—
Tidal volume = 700 ml.	Calculated	—	0.51	1.5	—	—
	Observed	—	0.54	1.5	—	—

In Table II a similar comparison is made with the experimental results of Bracken & Sanderson (1955). The agreement here is not nearly so good. Again the discrepancies are greatest in the region where the fresh gas flow-rate just eliminates rebreathing in the absence of longitudinal mixing, namely 4 l./min. Their greater magnitude can probably be attributed to the more thorough longitudinal mixing allowed by the larger dead space (300 ml. instead of 150 ml.).

TABLE II. COMPARISON OF CALCULATED VALUES OF MEAN PERCENTAGE OF CARBON DIOXIDE INSPIRED IN SYSTEM A WITH THOSE OBSERVED BY BRACKEN & SANDERSON (1955)

Fresh gas flow (l./min.)	10	8	6	4	2
Calculated	0	0	0	0	2.0
Observed	0.1	0.3	0.7	1.35	2.7

2. System E (" T-Piece " Arrangement)

In this system it is again assumed that a steady state exists and that there is no longitudinal mixing. It is also assumed that " square-wave " breathing occurs, that is, that respiratory flow is constant during inspiration and during expiration (see fig. 5).

Each tidal volume inspired into the alveoli consists of some expired gas from the dead space, some fresh gas from the anaesthetic machine and a volume of mixed gas drawn through the limb of the T-piece. If this last volume is greater than the volume of the limb, it will contain some air.

The magnitudes of these various volumes with square-wave inspiration are determined in the appendix. The work of Morton (1950) and Kavan & Haddy (1956), and some unpublished measurements made in this Department, suggest that the inspiratory waveforms of spontaneous respiration in anaesthetized patients are not such as to produce serious deviations from these values.

In expiration, fresh gas from the anaesthetic machine flows out of the system through the limb together with the patient's expiration. The proportions of fresh and expired gas remaining in the limb at the end of expiration, and the volume of expired gas which is inspired from there in the following breath, are determined in the appendix for the case of square-wave expiration. The available evidence indicates that, in anaesthetized patients, the flow rises to a maximum early in expiration and then declines—sometimes rapidly to zero, sometimes so slowly that there is still an appreciable flow at the end of expiration. Thus the square-wave expiration assumed here, in which the flow does not decline at all towards the end of expiration, is an extreme pattern. It results in an unduly high proportion of expired gas entering the limb towards the end of expiration and being rebreathed in the subsequent inspiration. Thus it is a cautious assumption.

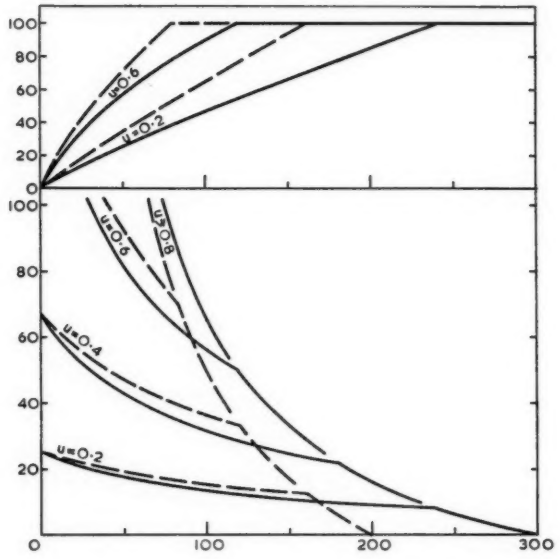
In the appendix the effects of the introduction of rebreathing are determined (with reference to the fresh gas flow-rate, the volume of the T-piece limb, and the respiratory parameters) in terms of, firstly, the increase produced in alveolar CO_2 when the respiration remains unchanged and, secondly, the increase in tidal volume needed to maintain the alveolar CO_2 constant. In both cases it is assumed that the CO_2 output is unchanged, which seems reasonable for moderate degrees of rebreathing. In practice, the extent to which the patient responds to inspired CO_2 will lie somewhere between the two limits depending on, amongst other factors, the degree of depression of the respiratory centre (see Spurrell, 1955). Equations are also derived for the proportion of anaesthetic mixture in the lungs when dilution with air occurs. All the results of the analysis are contained in equations (8) to (12) in the appendix, and their general pattern is indicated in figs. 2, 3 and 4.

The lower curves of fig. 2 show the way in which alveolar CO_2 increases as the fresh gas flow-rate is decreased (when the respiration stays constant). This is done for various T-piece limb volumes and two inspiratory/expiratory period ratios. Thus, for a ratio of 1 : 2, rebreathing is eliminated (0% increase in alveolar CO_2), with a fresh gas flow-rate of three times the minute volume. As the flow-rate is decreased below this, the alveolar CO_2 increases, at first slowly, then more and more rapidly, until a point is reached, depending on the volume of the T-piece limb, at which some air is inspired. Then further decrease of the fresh gas flow-rate produces only a limited increase in alveolar CO_2 but involves a substantial dilution of the anaesthetic with air (see upper curves of fig. 2).

Figures 3 and 4 show similar results in terms of the increase in tidal volume needed to maintain the alveolar CO_2 constant. Again the percentage of anaesthetic mixture in the lungs is also shown. Figure 3 is for a dead space of 0.3 times the tidal volume in the absence of rebreathing and represents the use of an endotracheal tube—dead space 150 ml., tidal volume 500 ml., say, in an adult. If a face-mask is used, the dead space will be increased by about 150 ml., to a total of 300 ml., and the tidal volume will increase almost as much (Clappison & Hamilton, 1956), to nearly 650 ml. Its use is therefore represented by fig. 4 where the factor is 0.5.

Finally it should be stressed that, with the respiratory waveforms met in clinical practice, the increases in CO_2 or tidal volume will not be as great as those given in the graphs, although the extent of the dilution with air will not be materially affected. On the other hand, longitudinal mixing will decrease the "safety factor" in CO_2 accumulation and increase the air dilution slightly.

FIG. 2. EFFECTS OF REBREATHING IN SYSTEM E WHEN THE PATIENT'S RESPIRATION DOES NOT RESPOND TO THE CO_2 INSPIRED



Abscissae: fresh gas flow-rate as a percentage of the minute volume respiration

Ordinates:

Upper curves: percentage of anaesthetic mixture in lungs (remainder is air)

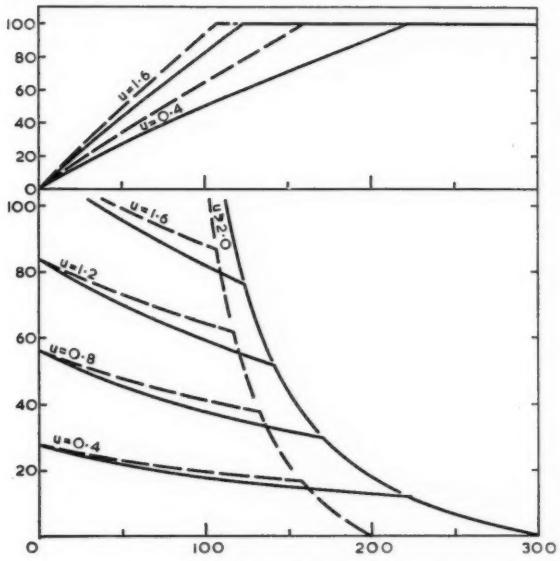
Lower curves: percentage increase in alveolar concentration of CO_2 over the value existing in the absence of rebreathing, assuming no change in the CO_2 output of the lungs. These ordinates can also be interpreted as the mean concentration of CO_2 in the effective part of the inspired tidal volume, expressed as a percentage of the alveolar concentration in the absence of rebreathing

Solid lines are for an inspiratory/expiratory period ratio of 1: 2, broken lines for a ratio of 1 : 1.

u = volume of T-piece limb expressed as a fraction of the effective tidal volume (tidal volume minus volume of dead space)

It can readily be shown that the ordinates for the lower curves of fig. 2 can also be interpreted as the mean concentration of CO_2 in the effective part of the inspired tidal volume, expressed as a percentage of the alveolar concentration in the absence of rebreathing. Thus the form of fig. 2 is essentially similar to that used by Inkster (1956) to display his main experimental results, and the general patterns are closely similar. A numerical comparison with one of his graphs shows that his figures for CO_2 and anaesthetic concentrations are somewhat lower than those predicted by the present theory. The respiratory waveform he used can be deduced from the diagram of his apparatus. In itself it does not explain the discrepancies but it may do so when considered in combination with the errors (mentioned by Woolmer & Lind, 1954) which arise from drawing the analysis sample at a constant rate during inspiration.

FIG. 3. EFFECTS OF REBREATHING IN SYSTEM E WHEN THE PATIENT'S TIDAL VOLUME INCREASES SUFFICIENTLY TO MAINTAIN THE ALVEOLAR CONCENTRATION OF CO_2 CONSTANT



Abscissae: fresh gas flow-rate as a percentage of the minute volume respiration in the absence of rebreathing

Ordinates:

Upper curves: percentage of anaesthetic mixture in the lungs (remainder is air)

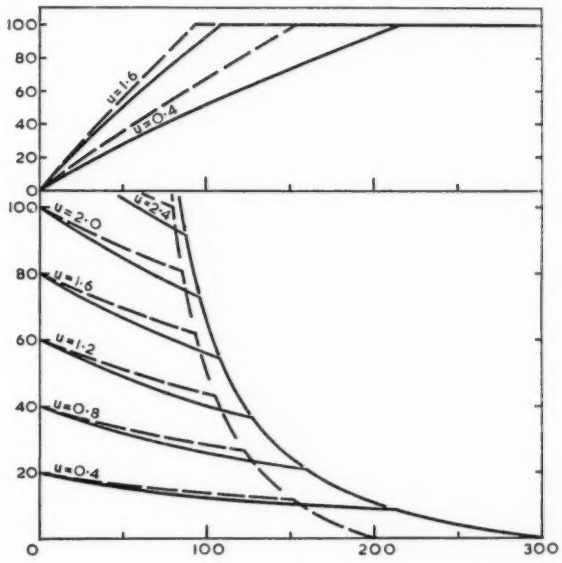
Lower curves: percentage increase in tidal volume needed to maintain alveolar concentration of CO_2 constant at the level obtaining in the absence of rebreathing, assuming no change in the CO_2 output of the lungs

Solid lines are for an inspiratory/expiratory period ratio of 1 : 2, broken lines for a ratio of 1 : 1.

u = volume of T-piece limb expressed as a fraction of the effective tidal volume (tidal volume minus dead space) in the absence of rebreathing

Volume of dead space = 0.3 × tidal volume in the absence of rebreathing

FIG. 4. EFFECTS OF REBREATHING IN SYSTEM E WHEN THE PATIENT'S TIDAL VOLUME INCREASES SUFFICIENTLY TO MAINTAIN THE ALVEOLAR CONCENTRATION OF CO_2 CONSTANT



Explanation as for fig. 3, except that the volume of the dead space = 0.5 × tidal volume in the absence of rebreathing

TABLE III. COMPARISON OF CALCULATED VALUES OF MEAN PERCENTAGE OF CARBON DIOXIDE INSPIRED IN SYSTEM E WITH THOSE OBSERVED BY WOOLMER & LIND (1954)

Fresh gas flow (l./min.)		10	7	5	3
Tidal volume = 400 ml.	Calculated	0.8	2.3	4.4	9.1
	Observed	1.3	2.7	4.6	7.5
Tidal volume = 500 ml.	Calculated	—	2.6	4.4	—
	Observed	—	2.8	4.3	—
Tidal volume = 700 ml.	Calculated	—	2.8	4.4	—
	Observed	—	2.8	4.0	—

In Table III Woolmer and Lind's experimental results are compared with values calculated according to the formulae in the appendix. (The diagram of their apparatus justifies assuming an inspiratory/expiratory period ratio of 1 : 1.) It will be seen that the agreement is good in most cases.

APPENDIX

The pattern of respiratory flow assumed in this analysis of system E is shown in fig. 5.

In inspiration, gas flows into the patient's lungs at a steady rate, $\dot{V}I$. This is made up of a flow-rate, $\dot{V}F$, of fresh anaesthetic gas from the machine and $\dot{V}I - \dot{V}F$ from the limb of the T-piece—unless $\dot{V}F > \dot{V}I$ when there is no rebreathing. Of the tidal volume, VT, only the first $VT - VD$ (where VD is the volume of the dead space) reaches the alveoli. Thus, of this, a volume

$$(VT - VD)\frac{\dot{V}F}{\dot{V}I} \qquad (3)$$

is fresh gas from the machine. The remainder,

$$(VT - VD)\frac{\dot{V}I - \dot{V}F}{\dot{V}I},$$

comes from the limb. If the volume of the limb, V_l, is greater than this the whole volume consists of mixed gas from within the limb. If, on the other hand,

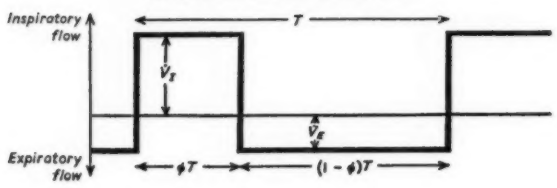
$$V_l < (VT - VD)\frac{\dot{V}I - \dot{V}F}{\dot{V}I} \qquad (4)$$

then only V_l of mixed gas enters the alveoli and the remainder

$$(VT - VD)\frac{\dot{V}I - \dot{V}F}{\dot{V}I} - V_l \qquad (5)$$

is air.

FIG. 5. RESPIRATORY FLOW PATTERN USED IN ANALYSIS OF SYSTEM E



For explanation of symbols see appendix

Consider first the case in which there is no dilution with air. In expiration, fresh gas flows into the limb at a rate $\dot{V}F$ accompanied by expired gas at a rate $\dot{V}E$. Thus the gas remaining in the limb for the next inspiration contains fresh and expired gas mixed in the ratio $\dot{V}F:\dot{V}E$. The volume of expired gas drawn from the limb into the alveoli on inspiration is therefore

$$(VT - VD)\frac{\dot{V}I - \dot{V}F}{\dot{V}I} \cdot \frac{\dot{V}E}{\dot{V}F + \dot{V}E}$$

In addition a volume, VD, of expired gas is drawn in from the dead space. This expired gas contains a fraction, FA_{CO_2}, of CO_2, where FA_{CO_2} is the fractional concentration of CO_2 in the alveoli. So the total volume of CO_2 inspired is

$$FA_{CO_2}\,VD + FA_{CO_2}(VT - VD)\frac{\dot{V}I - \dot{V}F}{\dot{V}I} \cdot \frac{\dot{V}E}{\dot{V}F + \dot{V}E}$$

At each respiration a volume of CO_2, $\dot{V}CO_2/f$, is added (where $\dot{V}CO_2$ is the CO_2 output of the lungs and f the frequency of respiration) and the whole expired as a fraction, FA_{CO_2}, of the tidal volume, VT. Therefore

$$FA_{CO_2}\,VT = \frac{\dot{V}CO_2}{f} + FA_{CO_2}\,VD + \\ + FA_{CO_2}(VT - VD)\frac{\dot{V}I - \dot{V}F}{\dot{V}I} \cdot \frac{\dot{V}E}{\dot{V}F + \dot{V}E}$$

From this, noting that $\dot{V}I\phi T = VT$ and $\dot{V}E(1-\phi)T = VT$ (see fig. 5) where T is the respiratory period (equals $1/f$), and writing $\dot{V}$ for the minute volume, ($\dot{V} = fVT$), it follows that

$$FA_{CO_2} = \frac{\dot{V}CO_2}{f(VT - VD) \cdot \dfrac{\dot{V}F/\dot{V}}{1 + (1-\phi)\dot{V}F/\dot{V}}} \qquad (6)$$

Now rebreathing is just eliminated when $\dot{V}F = \dot{V}I = \dot{V}/\phi$. Call the special value of FA_{CO_2} obtained with no rebreathing FAO_{CO_2}. Then it follows from (6) that

$$FAO_{CO_2} = \frac{\dot{V}CO_2}{f(VT - VD)} \qquad (7)$$

Then, if the introduction of rebreathing produces no change in $\dot{V}CO_2$ and no change in the parameters of the respiratory pattern, it follows from (6) and (7) that

$$\frac{FA_{CO_2}}{FAO_{CO_2}} = 1 - \phi + \frac{\dot{V}}{\dot{V}F} \qquad (8)$$

Alternatively if, in the presence of rebreathing, the tidal volume increases sufficiently to maintain FA_{CO_2} constant at FAO_{CO_2}, then, writing VTO and $\dot{V}O$ for the values of VT and $\dot{V}$ when there is no rebreathing—equation (7)—it is seen that

$$\frac{\dot{V}CO_2}{f(VTO - VD)} = \frac{\dot{V}CO_2}{f(VT - VD) \cdot \dfrac{\dot{V}F/\dot{V}}{1 + (1-\phi)\dot{V}F/\dot{V}}},$$

from which it can be shown that

$$\frac{VT}{VTO} = \frac{1 - \phi\left[1 - \dfrac{VD}{VTO}\right]}{1 - \dfrac{\dot{V}O}{\dot{V}F}\left[1 - \dfrac{VD}{VTO}\right]} \qquad (9)$$

Consider now the case when there is some dilution with air: that is, substituting for $\dot{V}I$ in (4), when

$$V_l < (VT - VD)(1 - \phi\dot{V}F/\dot{V}).$$

Then, by parallel reasoning, it can be shown that

$$FA_{CO_2} = \frac{\dot{V}CO_2}{f(VT - VD) - \dfrac{fV_l}{1 + (1-\phi)\dot{V}F/\dot{V}}},$$

$$\frac{FA_{CO_2}}{FAO_{CO_2}} = \left[1 - \frac{V_l}{VT - VD} \cdot \frac{1}{1 + (1-\phi)\dot{V}F/\dot{V}}\right]^{-1} \qquad (10)$$

and

$$\frac{VT}{VTO} = 1 + \frac{V_l/VTO}{1 + (1-\phi)\dot{V}F/\dot{V}} \qquad (11)$$

To determine the extent of the dilution with air it should be noted that, in the steady state, when anaesthetic equilibrium exists, the ratio of fresh anaesthetic to fresh air which is inspired into the alveoli at each breath must equal the ratio of "stale" anaesthetic to "stale" air which is expired from the alveoli and hence to the ratio existing in the alveoli. Thus, if x and y are the volumes of fresh anaesthetic and fresh air which are inspired, the fractional concentration of anaesthetic in the alveoli, $FA = x/(x + y)$. Now x is the sum of $(VT - VD)\dot{V}F/\dot{V}I$ from the anaesthetic machine (3) and $V_l\dot{V}F/(\dot{V}F + \dot{V}E)$ from the limb, while y is given by (5). Using these values, substituting for $\dot{V}I$ and $\dot{V}E$, and simplifying, it follows that

$$FA = \frac{\left[\dfrac{V_l}{VT - VD}\right]\left[1 - \dfrac{1}{1 + (1-\phi)\dot{V}F/\dot{V}}\right] + \phi\dfrac{\dot{V}F}{\dot{V}}}{1 - \dfrac{V_l}{VT - VD} \cdot \dfrac{1}{1 + (1-\phi)\dot{V}F/\dot{V}}} \qquad (12)$$

If respiration does not change in response to rebreathing, the "normal" values VTO and $\dot{V}O$ are used for VT and $\dot{V}$. If the tidal volume increases so as to maintain FA_{CO_2} constant at FAO_{CO_2}, then the values of VT and $\dot{V}$ must be derived from (11).

Equations (8) to (12) contain the information from which the graphs of figs. 2, 3 and 4 are plotted.

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AN INVESTIGATION INTO DEATHS ASSOCIATED WITH ANAESTHESIA

G. EDWARDS

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1 The investigation
2 Findings
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In Great Britain inquiries into deaths arising from or associated with anaesthesia have usually been limited in scope. Each year the Registrar-General correlates the findings recorded in death certificates; but the information published regarding deaths associated with anaesthesia is unsatisfying to the anaesthetist, who wishes to know not merely the age and sex of the patient and the nature of the anaesthetic used, but has also in mind the exact clinical condition of the patient and the manner in which the anaesthetic drugs were used, their effects, and also the results of the surgical measures employed. Without such detailed information a diagnosis of death due to anaesthesia can only be hazarded, and in most cases must depend upon the integrity of the anaesthetist concerned when describing the incident and upon the knowledge that the coroner and his pathologist possess of the expected course of events in similar circumstances. It is only rarely that the results of a post-mortem examination alone provide evidence of death due to anaesthesia. Even for comparison from year to year the Registrar-General's figures are not of much help, as they do not allow for the increasing age of the population, for changes in disease patterns, or for alterations in surgical and anaesthetic techniques.

More definitely clinical accounts have been published in our medical journals from time to time by individual anaesthetists; these have been limited to the reporting of a small number of cases occurring in one man's practice or to the assessment of series taken from the reports of one hospital or one group of hospitals. Just before the Second World War the London County Council called for reports on all deaths associated with anaesthesia which occurred in its hospitals: this widened the field of inquiry, but unfortunately the details asked for were still very meagre.

1. The Investigation

At the Annual General Meeting of the Association of Anaesthetists of Great Britain and Ireland held in October 1949, a plea was made for a more extensive approach to the problem, and as a result of that plea a scheme was evolved for the collection of full clinical and pathological data in cases where administration of an anaesthetic was accompanied or followed by death. It was stressed that the whole inquiry should be on a purely voluntary and absolutely confidential basis, the names of both patient and anaesthetist being suppressed. A questionnaire was devised and was carefully scrutinized and amended by the Council of the Association. Six copies of this questionnaire were sent to the Senior Anaesthetist of each Hospital Group in the National Health Service, together with an explanatory letter and an appeal for co-operation. It was also asked that the submitted returns should be signed by the Senior Anaesthetists: this was with the intention of assuring that the data submitted would be properly presented. The Council appointed a Committee of four anaesthetists to consider the reports as they came in, to correlate the findings and to make reports in what form and at whatever intervals seemed to be appropriate. The headings of the questionnaire are here reproduced (see fig. 1). Together with the space necessary for the replies, they occupy both sides of a foolscap sheet.

The questionnaire devised for the investigation was well received, and within a short time four reports a week on an average were returned to the Association. Each was registered and acknowledged, and a replacement form was sent back. Four typescript copies of the report on each case were made and were distributed—one to each member of the Committee, who made notes on points of significance and formed an opinion on the mode of death. At intervals the Committee met and discussed each return in detail. The facts recorded on the returns were indexed on a series of "punch-cards" for rapid cross-reference. The agreed opinion as to the type of fatality was also noted.

The investigation was planned in an attempt to obtain objective and detailed statements of the facts from the anaesthetists concerned. The actual causes of death, rather than their numerical incidence or frequency relative to a particular anaesthetic drug or sequence of anaesthesia, were wanted. The cloak of anonymity and the confidential nature of the inquiry were essential to allay any suspicion that the Association's Committee might appear to lay the "blame" for a death under anaesthesia on any person. There were, in fact, no reasons why the doctor concerned should not give a frank account of the proceedings.

The wealth of information furnished in most cases enabled the Committee to decide on the probable cause of death. The task was made easier by the close similarity of many of the descriptions provided. Indeed, when the person concerned was willing to co-operate at all, this simple questionnaire most often supplied all the information that could reasonably be expected, and certainly enough to ensure a measure of success in attaining the principal objective of the inquiry—the common causes of mortality due to anaesthesia.

When the general results of the investigation are considered, it must be accounted a disadvantage not to have known the status, experience or training of the doctor concerned in each case. In our opinion, the advantage of complete anonymity more than compensated for the absence of this knowledge.

2. Findings

Quite early in the inquiry the Committee noted the frequency with which episodes of regurgitation and vomiting were recorded. These appeared in so large a proportion of the

cases that two of the Committee undertook an immediate and detailed assessment of the question. Their observations were published in *Anaesthesia* (Morton & Wylie, 1951). When 400 forms had been examined, an interim report was submitted to the Association (Committee on Deaths Associated with Anaesthesia, 1952). An account of the clinical data recorded for cases in which deaths had been associated with anaesthesia accompanied by hypotensive techniques appeared in 1953 (Committee on Deaths Associated with Anaesthesia, 1953). In view of the small number of these cases and of the newness of the technique, it was not considered reasonable to make any general observations on the question nor to put forward any recommendations. Another short paper (Committee on Deaths Associated with Anaesthesia, 1955) was concerned with the cases reported in which death occurred during or after anaesthesia for tonsillectomy or adenoidectomy. One of the Committee (Pask, 1955) examined the question of inadequate ventilation and consequent carbon-dioxide retention, using clinical data from the reports.

In July 1956 the Committee's Report on 1,000 cases of death associated with anaesthesia was published by the Association (Edwards, Morton, Pask & Wylie, 1956).[1] This Report contains the inferences drawn from the clinical accounts submitted over five-and-a-half years by anaesthetists working in hospitals scattered all over Great Britain and Ireland, and it is reasonable to think that the evidence submitted may be taken to give a representative picture. The Committee classified the cases under eight headings :

i. Cases where it was reasonably certain that death was caused by the anaesthetic agent or technique of administration or in other ways coming entirely within the anaesthetist's province
ii. Similar cases, but in which there was some element of doubt as to whether the agent or technique was entirely responsible for the fatal result
iii. Cases in which the patient's death was caused by a combination of the surgical and anaesthetic techniques involved
iv. Cases entirely referable to surgical technique, e.g., uncontrolled haemorrhage
v. Inevitable deaths, e.g., cases of severe general peritonitis, but in which the anaesthetic and surgical techniques were apparently satisfactory
vi. Fortuitous deaths, e.g., those due to pulmonary embolism
vii. Cases that could not be assessed despite the inclusion of a considerable amount of data
viii. Cases on which an opinion could not be formed on account of inadequacy of the data

The Committee found that 589 of the 1,000 cases came into the first three categories.

After classification of the cases by the site of operation, by age and sex, by the pre-operative condition of the patient, by his chances of complete recovery, and by the stage of proceedings at which mishap took place, the Report goes on to discuss the various particular factors which appear to have led to disaster. It does so under the following headings:

i. Regurgitation or vomiting
ii. Obstetrics

[1] Copies of this Report are obtainable from the Association of Anaesthetists, 45 Lincoln's Inn Fields, London, W.C.2 (1s. post free).

FIG. 1. PLAN OF QUESTIONNAIRE USED FOR INVESTIGATION INTO DEATHS ASSOCIATED WITH ANAESTHESIA

STRICTLY CONFIDENTIAL NUMBER_____

RECORD OF DEATH ASSOCIATED WITH ANAESTHESIA

Only for the information of the Research Department, Association of Anaesthetists of Great Britain and Ireland, 45 Lincoln's Inn Fields, London, W.C.2

This is a scientific investigation, so please be as accurate as possible. If in doubt on any point, please say so

1 NAME OF HOSPITAL
PATIENT'S HOSPITAL No. Age Sex
Approximate weight
ADMINISTRATION Place Date Time
DEATH Place Date Time

2 CLINICAL DIAGNOSIS
CONDITION OF PATIENT BEFORE ADMINISTRATION
TEMPERATURE PULSE
RESPIRATION B.P. /
(a) GENERAL
(b) CIRCULATORY
(c) RESPIRATORY
(d) URINARY
(e) OTHER SYSTEMS

3 OPERATION (a) PROPOSED
OR (b) *PERFORMED*

4 SPECIAL PRE-OPERATIVE TREATMENT (e.g., *gastric lavage, glucose insulin, breathing exercises*)

5 PREMEDICATION
Time of giving Satisfactory/Unsatisfactory
METHOD OF INDUCTION
METHOD OF MAINTENANCE

6 APPROXIMATE TIMES OF
(a) starting induction
(b) starting operation
(c) ending operation
(d) first untoward signs
(e) respiratory failure
(f) circulatory failure
(g) cessation of restorative efforts

7 DETAILED ACCOUNT OF (a) ADMINISTRATION and (b) RESTORATIVE METHODS
Please attach copy of chart of blood pressure, pulse, etc., if available

8 SUMMARY OF POST-MORTEM FINDINGS (*please attach copy of pathologist's report, microscopical and macroscopical*)

9 IF INQUEST HELD, CORONER'S VERDICT AND COMMENTS

10 REMARKS

Signature of Senior Anaesthetist of Hospital Group _____

iii. Circulatory failure immediately following intravenous barbiturate injection
iv. Post-operative respiratory obstruction due to pharyngeal relaxation
v. Chloroform
vi. Trichloroethylene
vii. Apparatus
viii. Pre-operative upper respiratory tract obstruction
ix. Endotracheal anaesthesia
x. Convulsions
xi. Respiratory obstruction due to aspiration of blood during or after nose and throat operations
xii. Antidotes, analeptics and stimulants
xiii. Induced hypotension
xiv. Overdosage
xv. Underdosage
xvi. Inadequate ventilation
xvii. Epidural analgesia
xviii. Spinal analgesia
xix. Sudden death in children
xx. Pre-medication
xxi. Techniques associated with suboxygenation
xxii. Bronchospasm
xxiii. Inadequate resuscitation

It was noteworthy that in a large number of cases the cause of death was quite clear, and that in the majority of these there had been undoubted departures from accepted practice. This unsatisfactory state of affairs must be seriously considered by those responsible for undergraduate and postgraduate teaching. Of the 589 cases in which anaesthesia played some part as a cause of death, 110 were due to vomiting or regurgitation, and 107 to circulatory failure immediately following the intravenous barbiturate injection; in addition there were many smaller groups where the cause of death was equally obvious.

In a few cases, however, although death was undoubtedly due to anaesthesia, the mechanism was not completely explicable: for example, sudden death during trichloroethylene anaesthesia or following the intravenous injection of atropine and neostigmine. Further detailed reports of such rarer and obscure types of fatality would be of value, though ad hoc investigations may be necessary before they can be completely elucidated.

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Book Reviews

Anaesthetic Accidents: the Complications of General and Regional Anaesthesia

V. Keating. London: Lloyd-Luke (Medical Books) Ltd., 1956. vii + 261 pages; 13 figures. 22 × 14 cm. £1 5s.

A quick perusal of the contents page of this book gives an illuminating picture of the multiplicity of troubles that can beset a patient during anaesthesia: complications that range from the immediately fatal to the agonizingly prolonged, such as those that occasionally follow spinal analgesia, and, between these extremes, many minor difficulties that do little more than upset an otherwise tidy convalescence for the patient. This book is a measure of the interest that anaesthetists should and do take in this aspect of their specialty, rather than an indication that such complications are on the increase. As Dr Keating says in his preface, the attitude of many of the professional colleagues of anaesthetists to anaesthesia can be described as nonchalant. It is easy to forget that the anaesthetic may be a much greater hazard than the operation.

Since anaesthesia is essentially the practical application of basic science, it is not surprising to find that this work is approached from such an angle. The author sensibly describes normal physiology in order to underline the effects of anaesthetic drugs and techniques, and he bases his work on an extensive literature. Moreover, he is fortunately not afraid to make use of his own wide experience—a fact which adds weight to the wealth of information presented—and to be didactic when dealing with treatment. This is a very helpful and well-produced book.

W. D. Wylie

Inhalational Analgesia in Childbirth

E. H. Seward & R. Bryce-Smith. Oxford: Blackwell Scientific Publications, 1957. viii + 58 pages; 11 figures. 19 × 13 cm. 7s. 6d.

Analgesia for women in labour has been a subject of heated discussion for over 100 years. Although we no longer argue about its advisability there is much difference of opinion as to drugs and methods.

With the many agents available the main emphasis of teaching must be laid upon simplicity. In their monograph Seward and Bryce-Smith have addressed themselves mainly to midwives, and have even more rightly striven after the utmost directness of description and direction. They have, however, sometimes stressed the ideal rather than the strictly practical: for example, in a busy maternity unit where a few harassed midwives are coping with up to half a dozen labours at once, it is unrealistic to suppose that they will be free to time contractions accurately with the aim of instructing the patient better in the use of the apparatus.

The numerous indications, the contra-indications and the difficulties, that apply to the use of nitrous oxide or trilene and air mixtures are, of necessity, included. They serve, however, to emphasize that only by the constant attention of an anaesthetist interested in this type of work can a maternity unit hope to offer all its patients a truly adequate analgesia service. The "indifferent attendant" is listed as the last—instead of the first—of eight causes of failure.

This short book is very readable, and the eleven illustrations conform to the standard of excellence set by other publications coming from the Nuffield Department of Anaesthetics. The 56 pages will be read with advantage by all midwives and by all doctors who practise midwifery.

David J. Coleman

Lumbar Puncture and Spinal Analgesia

Robert Macintosh. 2nd ed. Edinburgh: E. & S. Livingstone Ltd., 1957. viii + 142 pages; 111 figures. 22 × 15 cm. £1 5s.

This most practical book continues the basic and simple approach to local anaesthesia established by the others from the same stable. That a second edition has been found necessary is witness to the demand and to the perfectionist aims of the author. In the preface to this edition Sir Robert Macintosh states: "A number of small alterations and improvements have been made in the script of this second edition. The original 110 illustrations, too, have been carefully revised and minor inaccuracies eliminated."

Do not seek herein any high-powered scientific discussion on the physiological and pathological effects of spinal anaesthesia. Certainly there is no pharmacological discussion of the various drugs that the spinal or epidural anaesthetist may use. The nearest approach to this is the pictorial description of experiments to illustrate the behaviour of solutions injected into the subarachnoid space, and an eminently practical discussion of the " reality " of the hypobaricity [1] of one in 1,500 solutions of Nupercaine. Incidentally, the continued use of proprietary names must be deplored.

Any discussion of the physiological effects of spinal anaesthesia is, strangely enough, to be found in a chapter entitled " Illustrations". Although this point must be considered as a deficiency in a monograph on spinal anaesthesia, the excellence of the anatomical illustrations makes up for a lot and the parts are unforgettably displayed. The advice given on every page of the book is that garnered from many years of great practical experience and matured by down-to-earth wisdom.

This is a book the reviewer would insist all young anaesthetists absorb despite the weighty and more scientific tomes that are available. It is to be compared in its field with A. E. Guedel's *Inhalation anaesthesia* (1937).

T. Cecil Gray

[1] The author classifies spinal anaesthetic solutions " . . . in three groups as hyper-, iso-, or hypobaric, depending on whether they are heavier, of equal weight, or lighter than cerebro-spinal fluid."—Ed.

Fluid Balance in Surgical Practice

L. P. Le Quesne. 2nd ed. London: Lloyd-Luke (Medical Books) Ltd., 1957. vii + 140 pages; 42 figures. 22 × 14 cm. £1

The popularity of the first edition of this book was well deserved; I am sure this second edition will be equally well received. Mr Le Quesne is a surgeon writing for the benefit of surgeons. After a short introductory chapter on units of measurement, and the normal metabolism of water and electrolytes, there is a lucid and brief account of the effects of surgery on water, electrolyte and nitrogen balances. This is followed by an account of the most practical means whereby these can be controlled. It is, however, a sad reflection on the lag in the spread of knowledge, that in 1957 it is still necessary to explain the use of milli-equivalents.

Much of the work on the post-operative water and electrolyte changes has been performed by the author himself, in association with Dr A. A. G. Lewis. This is evident in the text which is interesting and factual throughout. One of the main themes of the book is that, although there are many and complex electrolyte disturbances, they can, if renal function is normal, usually be treated by the administration of a standard amount of salt, potassium and water; or, in other words, that the basis of most intravenous therapy is to give an excess and rely on the kidneys to decide what to keep and what to reject. Perhaps this principle is intuitively understood by all who give intravenous fluids, but it is not often appreciated at a conscious level. In surgical cases it is particularly important, for anaesthesia, trauma, morphia, etc. cause changes in renal function which increase the risk of overloading. The author repeatedly points out the existence of this danger, and emphasizes that " . . . in any case of doubt it is wise to restrict rather than to increase the intake." It is, of course, implicit that such doubts can only arise if there is some awareness of the patient's pre- and post-operative renal functional state; if only as regards the amount of urine that is being passed. It is interesting to note that post-operative water intoxication was first described 22 years ago.

There are two small points which might be adjusted in another edition. First, the remarkable statement that a person with normal renal function will concentrate up to a specific gravity of 1,025 after only 14 hours' fluid deprivation (p. 127–8). The second is the confusion on p. 90 where the reader is advised to treat acute renal failure with the daily administration of 1,000 ml. of water and 2,500 calories, including 100 ml. arachis oil; a few lines further down, however, it is pointed out, quite rightly, that the fat usually causes vomiting, and that it is better to restrict the water intake to 600–800 ml. per day.

One of the new features of this edition is a section on fluid balances in children, by Dr B. Webb; it is only nine pages long and is first-class. Finally, the book ends with a chapter on such technical considerations as the site where the intravenous needles should be placed, the type of needle to be used, how to prevent thrombosis. This chapter alone should be compulsory reading for all students.

Hugh de Wardener

World Medical Periodicals

2nd ed. Prepared by *L. T. Morton* under the auspices of a Committee jointly sponsored by the World Medical Association and the International Union of the Medical Press. New York: World Medical Association, 1957. xxxiii + 340 pages. 24 × 16 cm. £1 10s. or $6.00 (by post £1 11s. 6d. or $6.25)

The second edition of this important reference book includes titles of journals of medicine, pharmacy, odontology and veterinary medicine which were in existence in 1957, and also titles of well-known journals which have ceased publication since 1900, but to which frequent reference is made in current bibliographies. The titles of 4,841 journals, including the principal international abstracting journals and indexes, are listed, and in this edition the names and addresses of publishers are also given. There is an index of periodicals by country, and one by subject. The abbreviation of titles follows the code of rules used by the *World list of scientific periodicals*, modified by the recommendations of the International Organization for Standardization (*ISO Recommendation R4*).

This publication will be useful to medical workers, editors, publishers and librarians. Interleaved copies can be supplied on special order to those who may wish to annotate the book, to record changes as they occur, or to use the book as an index to their own holdings.

O.M.H.

How to Use a Medical Library: a Guide for Practitioners, Research Workers and Students

Leslie T. Morton. 3rd ed. London: William Heinemann Medical Books Ltd., 1957. vii + 53 pages. 19 × 12.5 cm. 7s. 6d.

Directory of Medical Libraries in the British Isles

Compiled by a sub-committee of the Medical Section of the Library Association. London: The Library Association, 1957. viii + 91 pages. 22 × 14 cm. 13s. + 6d. postage

Medical literature today has attained enormous proportions, but, as the author of this book says: " Careful indexing has made this vast and ever-growing accumulation easily accessible . . . " The book is intended to supply a key to medical literature, for the research worker who needs to make a systematic examination of abstracts and indexes concerning his own subject and for the general practitioner who will supplement his chosen medical journals with a good abstracting journal and an occasional glance at indexes.

A brief account is given of the most important works of information and reference, and the location of books and periodicals is discussed. There is a chapter on the compilation of medical bibliographies and one on abstracting services which, in this edition, includes a list of the principal abstracting journals. Of particular interest to the worker in Britain is a chapter giving details of the chief British medical libraries.

This chapter has been brought up to date with the help of the *Directory of medical libraries in the British Isles*. The first section of this publication lists the principal medical libraries, and gives brief notes on their history, the type and number of their holdings and special collections, and other details of staff and services provided. The second section lists hospital staff libraries and others giving facilities restricted to a small group of users. Both sections are arranged on a geographical basis.

O.M.H.

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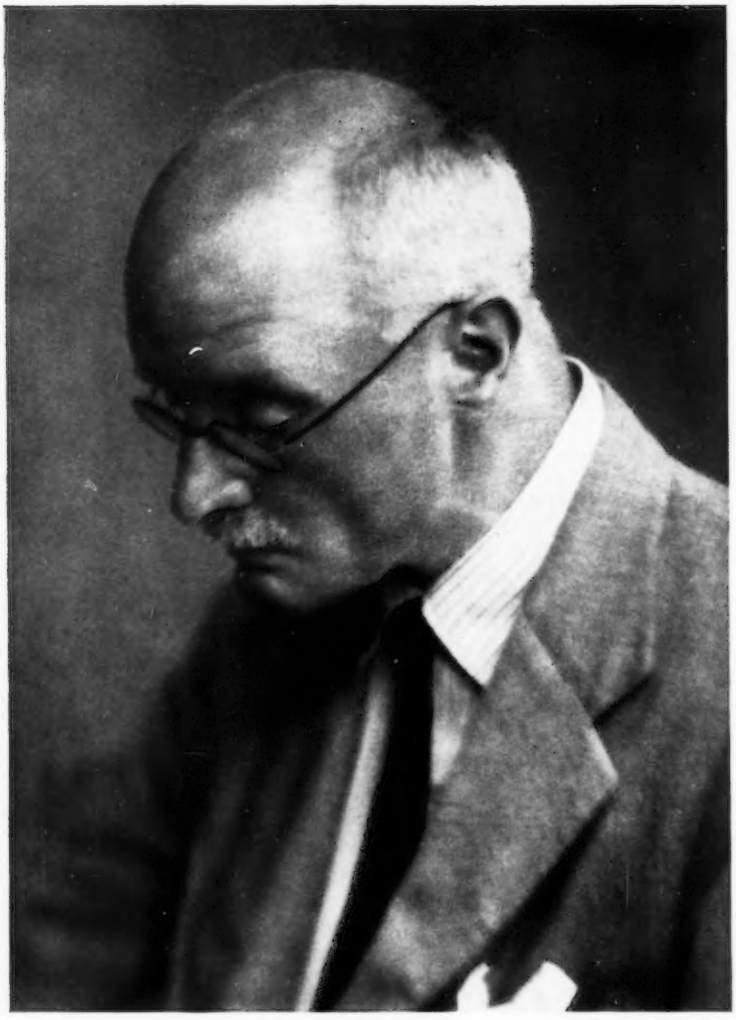

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SIR ERNEST KENNAWAY
1881–1958

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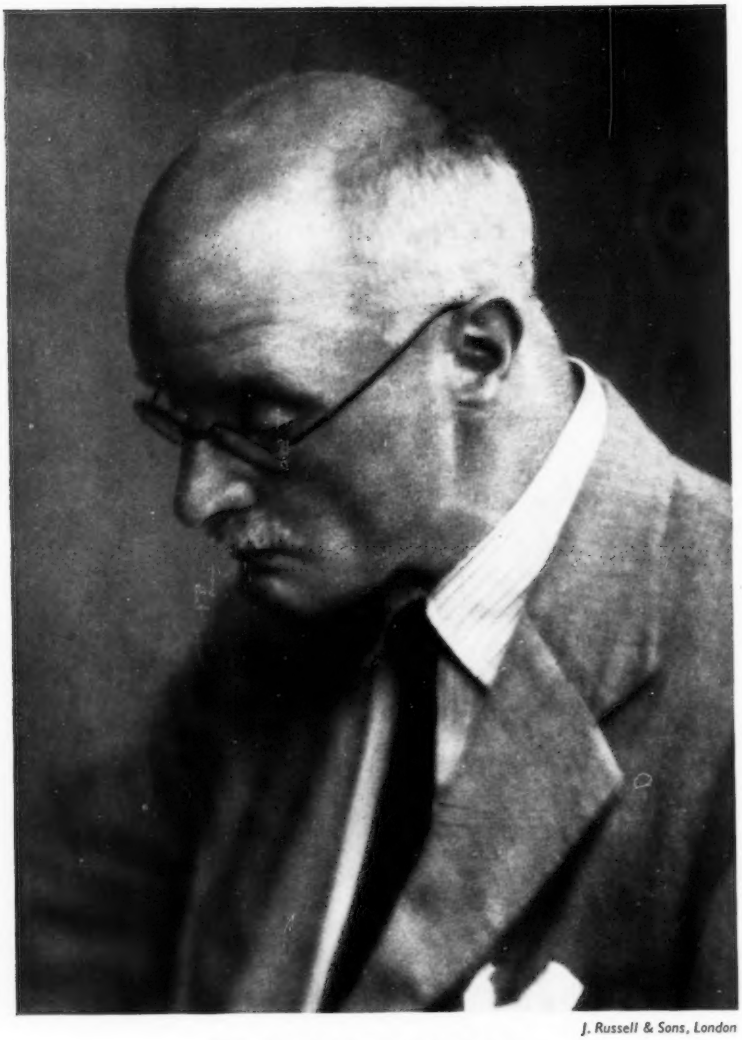
Causation of Cancer: Introduction

This issue of the *British Medical Bulletin* is devoted to cancer causation. During its preparation Sir Ernest Kennaway, who had contributed so massively to the growth of this subject, died peacefully in his sleep at St Bartholomew's Hospital on the first of January of this year, and it was decided that a symposium on the causation of cancer would not be complete without a tribute to him; hence this Introduction has taken its present form. Sir Ernest's death brings to an end a whole era in cancer research, one which was inaugurated in the same St Bartholomew's Hospital two hundred years before; and we can imagine a salute to him from the shade of Sir Percivall Pott.

Ernest Laurence Kennaway was born on the 23 May 1881. As a scholar of New College, Oxford, he took a first class in the final honours school of natural science in 1903. Proceeding to the Middlesex Hospital with a university scholarship, he graduated B.M., B.Ch., in 1907, and D.M. in 1911. After working at the Lister Institute and at University College, London, he then



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successively became Demonstrator of Physiology at St Thomas's Hospital, Hulme Research Student at Brasenose College, Oxford, and Demonstrator of Physiology at Guy's Hospital (1909–14). In 1911, he was awarded a Radcliffe Travelling Fellowship by the University of Oxford, and studied at Heidelberg and at Munich.

He joined the staff of the Cancer Hospital, London, as Physiological Chemist in 1921, and in due course became Director of its Research Institute (now the Chester Beatty Research Institute), in succession to Dr Archibald Leitch; and was also elected Professor of Experimental Pathology in the University of London. He retired from the staff of the Royal Cancer Hospital in 1946, becoming professor emeritus, and continued his researches—almost to the day of his death—at St Bartholomew's. Here he applied himself especially to the study of exogenous factors in the causation of cancer in man. Not long before his death he had undertaken, and was able to complete—in collaboration with A. J. Lindsey—a paper on this aspect of lung cancer, which appears in these pages, and greatly enhances their value.

In a long life he accumulated many honours—the William Julius Mickle Fellowship of the University of London, the first award of the Anna Fuller Memorial Prize (jointly), the Garton Prize of the British Empire Cancer Campaign, the Walker Prize of the Royal College of Surgeons of England, the Baly Medal of the Royal College of Physicians of London, the Osler Memorial Medal of the University of Oxford, and an honorary fellowship of New College—among others. He was elected Fellow of the Royal Society in 1934 (and a Royal Medallist in 1941), and of the Royal College of Physicians in 1937; and received the honour of knighthood in 1947.

Not all Sir Ernest's scientific life was spent in the cancer field, and we must not forget a whole series of papers, from about 1910 to 1920, which revealed the breadth of his knowledge and interests. Particular contributions in this period dealt with such a variety of topics as purine metabolism in hibernating animals, the effects of section of the spinal cord upon temperature and metabolism (with M. S. Pembrey), the excretion of acetone bodies, the antigens of the Wassermann reaction (with C. H. Browning), nitroclupein (with A. Kossel), and German chemical nomenclature. All these gave a taste of things to come, and a communication to the *Guy's Hospital Gazette* of 1912 is entitled " Some notes on growth ".

Certainly it is upon his achievements in the sphere of malignant disease—and especially in that of chemical carcinogenesis—that his name and fame are based and will endure. Curiously, an early paper in this phase, on the experimental production of cancer by arsenic (with A. Leitch, *Brit. med. J.* 1922, **2,** 1107), was one on which he set much less store in later years. But this subject continued to exercise him, and a further contribution, on the effect of arsenic upon some oxidation-reduction systems (with G. Barry and E. Bunbury, *Biochem. J.* 1928, **22,** 1102), is still much consulted, and may indeed prove to have greater significance than was appreciated at that time.

In the early 1920's, great efforts were under way to elucidate the nature of the cancer-producing agent in tar and pitch, following the production of undoubted cancer through application of coal-tar to the rabbit skin by Yamagiwa and Ichikawa in 1915, and in the mouse by Tsutsui in 1918. Between 1921 and 1926, Bloch in Zürich had found that the active substance was concentrated in the higher boiling fractions as a neutral compound, free from nitrogen, arsenic or sulphur, capable of forming a stable complex with picric acid, and probably belonging to the class of cyclic hydrocarbons. In 1924–25, Sir Ernest obtained virtual proof of this, having succeeded in producing carcinogenic tars artificially, by the pyrolysis of many organic materials, and by passing acetylene and isoprene with hydrogen through heated tubes. The tars obtained at successively higher temperatures (450–1,250° C.) showed an ascending order of potency; and these results could be explained by assuming that acetylene was a common decomposition product, and that the carcinogenic material was actually formed from acetylene. There gradually emerged the strong general conclusion that the carcinogenic agent was indeed a complex hydrocarbon of aromatic type. In all this work, Sir Ernest ever acknowledged the value of Berthelot's classical paper of 1866 describing for the first time the production from acetylene of these very kinds of molecule.

We are fortunate indeed that he left his own characteristic account of these and later developments (*Brit. med. J.* 1955, **2,** 749), setting forth their history, and making clear the part played by those in his school at the Cancer Hospital: W. V. Mayneord's examination of the fluorescence spectra of the carcinogenic tars, and the discovery of their special qualities (1927); the development by I. Hieger of this vital clue; the discovery that 1 : 2-benzanthracene gave a spectrum similar to that of the carcinogenic mixtures; J. W. Cook's studies of the

benzanthracene homologues; and by no means last, Sir Ernest's own indebtedness to the help of his assistant, Frank Goulden. About this time, Erich Clar had just published methods for the synthesis of certain benzanthracene hydrocarbons. These compounds were tested for carcinogenic activity by Kennaway and Hieger in 1929–30, who soon observed positive results with 1:2-5:6-dibenzanthracene: this was the first pure compound manifesting pronounced carcinogenic properties, and one cannot forget the satisfaction and modest pride which Sir Ernest obtained from the result. The fluorescence spectrum—in Sir Ernest's words "the single thread that led all through this labyrinth"—was also used by Hieger in the concentration of the agent from coal-tar pitch, culminating in the isolation of a hydrocarbon which showed the characteristic spectrum, and which proved to be strongly carcinogenic (1933). Its properties suggested one of the two benzopyrenes, and these were synthesized by J. W. Cook and C. L. Hewett, who were then able to prove the identity of one of the compounds (3:4-benzopyrene), and the substance from pitch. No imagination is required to see what determination and skill Sir Ernest had contributed to this conclusion. Equally, he never failed to point out the influence of chance and good fortune on the course of these researches: for example, that "any further advance would have been difficult indeed if one at any rate of the carcinogenic substances in tar, namely, 3:4-benzpyrene, had not possessed this extraordinary property of blotting out with its own fluorescence that of other intensely fluorescent compounds, such as anthracene, in the complex mixture." All these, and many other results, which Sir Ernest alone inspired, are embodied in numerous papers (many of them classics) in the *British Medical Journal*, the *Journal of Industrial Hygiene*, the *Journal of Pathology and Bacteriology*, the *Biochemical Journal*, the *Journal of Hygiene*, the *American Journal of Cancer*, the *Yale Journal of Biology and Medicine*, *Nature*, *Cancer Research*, the *British Journal of Cancer*, and, most notably, in a celebrated series on the production of cancer by pure hydrocarbons, which appeared in the *Proceedings of the Royal Society* between 1932 and 1942.

These were thrilling days, not least from the recognition of a possibility that such compounds might play a part in the causation of "spontaneous" cancer. This followed the advances in sterol formulation due to Rosenheim and King in 1932, showing that many such naturally occurring compounds have somewhat similar condensed polycyclic systems. Also, J. W. Cook had drawn attention to evidence suggestive of an inherent tendency for certain sterol molecules to pass into carcinogenic derivatives. There is, as yet, no proof that changes of this kind take place, or do not take place, in vivo. Yet the possibility was one which continued to intrigue Sir Ernest, and led him to much further experimentation. Especially, he was able to confirm an observation of Ghiron, that the bile-acid deoxycholic acid is capable of producing connective-tissue tumours in mice. This result was of particular interest, since Wieland and Schlichting had already prepared from deoxycholic acid the hydrocarbon dehydronorcholene—a hydrogenated derivative of 1:2-benzanthracene,—from which (in 1933) in turn both Wieland and Dane, and Cook and Haslewood obtained, by dehydrogenation with selenium, the fully aromatic and powerfully carcinogenic hydrocarbon methylcholanthrene.

What undoubtedly emerged from almost thirty years of brilliant work was a satisfying and indeed entrancing correlation between chemical constitution and biological action, whereby the carcinogenic subclasses—benzophenanthrenes, dibenzophenanthrenes, chrysenes, pyrenes and benzanthracenes—could clearly be related to the parent phenanthrene, in a system suggesting dependence upon certain optimal features of molecular size, shape, substitution and chemical reactivity. But we must not minimize another result, namely the profound practical influence which was exerted upon the whole of cancer research, and indeed biology widely, from the mere availability of these potent carcinogens, so rendering possible numberless other investigations and additions to knowledge which could not have been attained without them.

In addition to his many preoccupations in the laboratory and in administration, Sir Ernest also found time to write papers on a great diversity of aspects, including occupational cancers, the serum proteins in malignant disease, the nitroprusside reaction in normal tissues and in tumours, the incidence of carcinoma of the lung and larynx, the carcinogenicity of azonaphthalenes, cancer of the liver in the African and American negro, and the social distribution of cancer of the scrotum. In a prophetic sentence written as long ago as 1851, Lebert (*Traité pratique des maladies cancéreuses*) suggested that the discovery of cancer-producing agents could come about only through the joint efforts of one accomplished in the anatomy and pathology of cancer and a chemist versed in science. This conjunction was certainly

reached when Sir Ernest joined forces with J. W. Cook. But in many ways he possessed all the necessary qualities in his own being—in his intellectual aptitudes, his chemical sympathy and knowledge, and in his unrivalled skill in applying them to physiology and pathology. His standards of evidence were rigorous and severe to the point of austerity. His experimental methods were simple, practical and decisive. He was much less interested in hypotheses and explanations and, although his own work had lain almost entirely in the chemistry of carcinogenesis, he remained completely open and free to accept any other mechanism or interpretation—infective, viral or otherwise—provided only that the facts were ascertained, and not an instant before. Added to all these gifts, he was a master of scientific expression and of English construction. He especially disliked the use of hypothetical or presumptive words or phrases, on the simple ground that so many things which " should " take place in this world, in fact don't.

We have lost a great seeker and searcher, a great discoverer, and scientific worthy. No one who worked with him can ever forget the part played by Lady Kennaway in every aspect of his life and contribution. Owing to her, he was able to defy infirmity, illness and accident, and to write a vast chapter in cancer research and chemical pathology, which nothing can diminish, or take away.

Although cancer research in Britain has lost its doyen, it must continue, and for many a day will be inspired by his memory and example. The present issue of the *British Medical Bulletin*—comprising papers by 27 contributors from 16 different centres—is the successor to one on chemical carcinogenesis which appeared in 1947 (*Brit. med. Bull.* **4**, 309–426). Inevitably, comparison is prompted between now and then. Certain developments are entirely fresh to this period, especially the discovery of a whole range of biological alkylating agents with powerful carcinogenic and mutagenic properties. In many other fields there has been steady advance, as for example in our knowledge of the biological effects of the carcinogenic amines, and of the behaviour of these substances in metabolism, and its significance. The period of eleven years has witnessed a great revival in tumour immunology, with considerable and unexpected strides in our understanding of transplantation immunity and immunogenetics. The nature of the autonomy of tumours at one extreme, and their hormonal or other dependence at the other, has been much clarified through numerous contributions in endocrine carcinogenesis. Many fresh proofs have been given of the power of the statistical weapon. Lastly, the advent of the nuclear age has not only brought startling and giant advances, but has also created innumerable problems of hazard and control, the end of which no man can see.

The main value of the biological alkylating agents has proved to be on the fundamental side rather than in therapy, as relatively simple reactive compounds with features suggestive, if not indeed indicative, of likely modes of action. A main problem is the chemical nature of the most significant cellular receptors with which they may combine. Among these, the deoxyribonucleic acid structure is certainly a prominent candidate, and the picture now emerging is one of mutation by cytogenetical loss, engendered by interference with the synthesis and function of this acid, and so possibly leading to the deletion of growth-controlling or regulatory proteins. The sphere of occupational and industrial medicine still continues to stimulate that of chemical carcinogenesis. Thus, special interest attaches to an investigation, under the aegis of the Medical Research Council and with the collaboration of the Institute of Petroleum, of the carcinogenicity of mineral oils and the discovery of condensed polycyclic thiophen derivatives in fractions of Kuwait oil. Again, much progress has been made in studies of the aromatic amines as carcinogens, a novel development being the discovery of activity in 4-aminodiphenyl (xenylamine) and certain derivatives. In the specialized biochemistry of cancer of the bladder, we now possess remarkably complete knowledge of the metabolic transformations of 2-naphthylamine, and of their significance for the carcinogenicity of this substance, or the lack of it, in various species; and a further quite unexpected turn has been given by the incrimination of tryptophan metabolites in the same disease. In the experimental induction of cancer of the bladder, technical progress has been made in the test of amines and their metabolites by direct application to the bladder epithelium. Especially, this has permitted systematic examination of a possibility that the active or causal metabolites might well be *o*-aminophenols. For the present, this hypothesis appears to remain an extremely valuable one for the prediction of compounds likely to be carcinogenic either to the experimental animal or to man. Another generalization, which has received much support, suggests that excretion as glucuronides, rather than as ethereal sulphates, is of special significance in determining the carcinogenicity

of the aromatic amines. For the alkylating carcinogens it has already been proposed that they could interrupt the synthesis of specific proteins by degradation of local sites in the macromolecule of deoxyribonucleic acid. For the carcinogenic hydrocarbons and azo dyestuffs, evidence had been adduced for the same kind of end-result, attained through protein-binding followed by deletion. From studies of the nitrogen balance and of the influence of dietary protein, there now comes a third possibility: that the primary action of certain carcinogens may be to prevent the utilization of amino acids for protein synthesis and growth, whilst at the same time the utilization of these substances for energy metabolism is unaffected. On a more biological level, continued investigation of the phenomenon of cocarcinogenesis has shown that while the multistage theory of cancer induction has required some modification, nevertheless it still commands support in principle. At the same time, other mechanisms (as alternatives to the so-called latent cell theory) are also being pursued, especially that which postulates an essential role on the part of the collagen and elastic fibres of the supporting connective tissue. Apart from questions of the modes of action, the number and variety of chemical carcinogens themselves continue to expand, and interesting additions in the special case of hepatocarcinogenesis are seen in dimethylnitrosamine, thioacetamide, and the pyrrolizidine alkaloids. There is now an immense range of chemical types endowed with carcinogenic potentiality. Inevitably they must operate through biochemical routes which initially are very different, and the great question still remains, whether, despite this, the ultimate and key alteration may not be chemically and genetically the same, if not in every case in detail, at least in biological principle.

Twenty-five to fifty years ago, a vast amount of effort was expended in the field of tumour immunity. Although the approach is certainly among the most logical, nevertheless the results without exception proved negative or inconclusive. The revival which has taken place in this sector over the past few years is in part due to the advent of new techniques even more subtle than the old, but also in part to new ideas, and especially to the great conception of acquired tolerance. It now appears that the carcinogenic process may indeed have a true immunological basis, the transformation perhaps being inaugurated through the binding of carcinogen to protein to yield an auto-antigen, reaction against which induces antigenic deficiency, through the loss of organ-specific "marker" or "recognition" antigens possibly of the nature of lipoprotein complexes. The reader cannot escape the similarity of this immunological interpretation to other theories—already mentioned—of biological mutation through loss and protein deletion. Indeed these conclusions, reached from so many different avenues of attack, are not merely mutually consistent but in fact convergent. At the same time, final proof is lacking and further evidence is still required, pending which other hypotheses must continue to be entertained, as for example that the growth of the cancer cell is due not to antigenic deficiency and the consequent failure of any immune response on the part of the body, but to an increase of cellular resistance to those immune mechanisms which would otherwise prevail.

The multifarious nature of cancer causation is illustrated by many other examples. Nowhere has ingenuity of experimental planning been more widely or more successfully applied than in endocrine carcinogenesis, the result being an impressive contribution to comprehension not only of the role of hormonal imbalance in the origin of neoplasia, but also of the cell's progression to true autonomy. In this period, the situation has been affected by advances in pituitary physiology, the gland now being regarded not so much as the master, but rather as the servant of the hypothalamus. Lastly comes that vast and unprecedented expansion in the field of radiation carcinogenesis, as part of the immense stimulus to radiobiology from atomic energy programmes whether peaceful or military. We recall that the carcinogenic action of ionizing radiations was recognized very shortly after the first medical application of *x* rays, and that it was confirmed experimentally as long ago as 1910. Notable among the biological responses to damage by nuclear and allied radiations is the induction of leukaemia, and now we face such vexed questions as, firstly, the recorded increase in the death-rate from leukaemia in many countries between 1940 and 1955, and the fraction of it (if any) attributable to environmental radiations; and, secondly, the nature of the dose-response relationship in radiation carcinogenesis, whether it be arithmetically proportional to dosage at all levels, as is believed to be true for genetical effects, or subject to a necessary threshold. If no experimentation has yet proved a linear relationship between tumour incidence and dose, the possibility of such cannot altogether be excluded. Whatever conclusions will ultimately be reached in these and similar problems there is no doubt of the contribution which has already been made by

modern radiobiology, one which will increasingly accrue in the future, towards elucidating cancer causation.

In closing this Introduction, it is a pleasure to acknowledge the endeavours of all who have made possible this number of the *British Medical Bulletin*, including our willing contributors. It must not be forgotten that a great part of the researches reviewed in these pages was carried out with the support of the British Empire Cancer Campaign and the Medical Research Council, and hence owes much both to the charitable public and to the State. I am sure the issue will be regarded (as can be claimed for its forerunner) as a milestone in the development of our subject. One's main impression of the eleven years between the two is of a certain movement towards greater accuracy of knowledge. The present number has exceptional timeliness, appearing as it does just before the Seventh International Cancer Congress—to be held in London this year—which will allow an appraisal of the state of cancer research in all its aspects. Among these aspects, the study of causation is central. There would seem to be no reason why the carcinogenic process should not ultimately be deciphered in chemical terms, so leading to precise understanding of the vital if elusive difference between normal and cancer cells—with all that this would mean.

Alexander Haddow

The publication of the symposium has been timed so as to precede the Seventh International Cancer Congress which takes place in London from the 6 to the 12 July 1958.

* * *

ACKNOWLEDGEMENT

Professor A. Haddow has acted as Chairman of the Committee which planned this symposium, and Professor E. Boyland as its Scientific Editor. We are most indebted to them both for all the help they have given in its production.

CHEMICAL CARCINOGENS AND THEIR MODES OF ACTION

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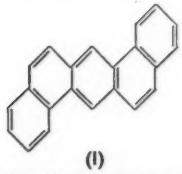
1 The carcinogenic hydrocarbons
2 Azo carcinogens
3 Aromatic amines
4 Polymers and plastics
5 Miscellaneous chemical agents
6 Alkylating carcinogens and mutagens
7 Interpretation and conclusions
References

1. The Carcinogenic Hydrocarbons

Through the death of Sir Ernest Kennaway, cancer research in Britain has lost its doyen, and it is not too much to say that his passing brings to an end a whole era in our subject. With others in his school, he was responsible (in 1928–29) for the discovery of the first pure chemical individual to manifest pronounced carcinogenic properties—1 : 2-5 : 6-dibenzanthracene (I); and for the ultimate identification (in 1933) of 3 : 4-benzopyrene (II) as the active substance in carcinogenic

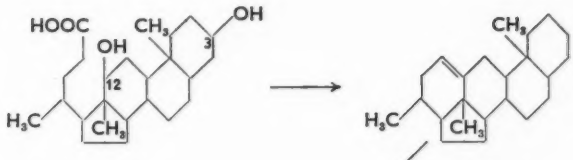


(I)
1 : 2-5 : 6-Dibenzanthracene

(II)
3 : 4-Benzopyrene

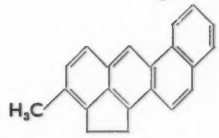
pitch. These were thrilling days, and the possibility that such hydrocarbons might play a part in the causation of " spontaneous " cancer was opened up by recognition, following the advances in sterol formulation due to Rosenheim and King in 1932, that many naturally occurring compounds have somewhat similar condensed polycyclic systems; and J. W. Cook had drawn attention to an inherent tendency for certain sterol molecules to pass into carcinogenic derivatives. In particular, the hydrocarbon dehydronorcholene (IV), prepared by Wieland and Schlichting from the bile-acid deoxycholic acid (III), is a hydrogenated derivative of 1 : 2-benzanthracene, and both Wieland & Dane (1933), and Cook & Haslewood (1933), obtained from it, by dehydrogenation with selenium, the fully aromatic and powerfully carcinogenic hydrocarbon methylcholanthrene (V).

Numerous other examples have meantime come to light, of the possible relationship of natural steroids to carcinogenic aromatic compounds, as in the Nes-Mosettig transformation (Nes & Mosettig, 1953), by the re-arrangement of dehydroergosteryl acetate (VI) to a hexahydroanthracene derivative (VII). Also, Gough & Shoppee (1953) point out that the



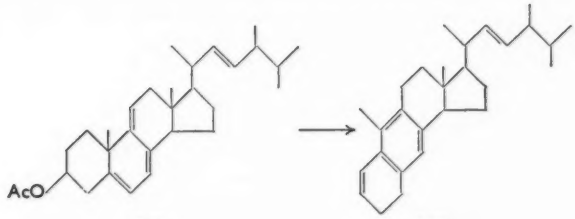
(III)
Deoxycholic acid

(IV)
Dehydronorcholene



(V)
20-Methylcholanthrene

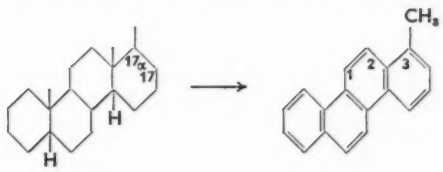
structural analogy between a cholanic acid and methylcholanthrene is paralleled by the even closer analogy between 17α-methyl-*D*-homoandrostane (VIII) and 3-methylchrysene (IX). Fieser (1954) has discussed similar aspects of the chemistry and biochemistry of cholesterol, in relation to the observation of Hieger (1946, 1947, 1949) that subcutaneous injection of cholesterol in lard solution into several hundred mice resulted in a low incidence of tumours at the injection



(VI)
Dehydroergosteryl acetate

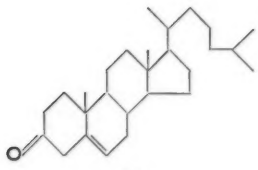
(VII)
1 : 2 : 3 : 4 : 7 : 8-Hexahydro-3′-(5 : 6-dimethyl-3-heptenyl-2)-2 : 10-dimethyl-1 : 2-cyclopentanthracene

site. It appeared to Fieser that cholesterol itself could hardly have the properties of a carcinogen with potency of even a low order, but that the tumours must have been initiated by some transformation product, or some unknown companion substance. Among the many individuals derived from cholesterol by oxidation, there was, according to Fieser, only one which met the reactivity requirements, namely, Δ^5-cholestene-3-one (X)—in his view the one substance derived from or related to cholesterol most likely to possess carcinogenic activity. From it he later obtained (Fieser, Greene, Bischoff, Lopez & Rupp,



(VIII)
17α-Methyl-D-homoandrostane

(IX)
3-Methylchrysene



(X)
Δ⁵-Cholestene-3-one

1955) 6β-hydroperoxy-4-cholestene-3-one, which, when administered to 32 mice in sesame oil, produced fibrosarcomata in 13. Finally, Inhoffen (1953) has extensively discussed the relationship of natural steroids to carcinogenic compounds, through the aromatization of sterols and bile acids. Although there is still no proof of the generation of methylcholanthrene from cholanic acid derivatives in vivo, many ingenious suggestions continue to appear of routes by which this or other carcinogens might so be formed. If the majority of chemists are doubtful or non-committal, the biologist cannot but be impressed by the fact that only an infinitesimal trace of the end-product would be required, in order very reasonably to account for a proportion of spontaneous tumours at least.

In spite of these intriguing possibilities and speculations, it soon became abundantly apparent that carcinogenic activity was by no means restricted to the polynuclear aromatic hydrocarbons, but was shared with a great range of chemical classes —benzacridines, benzocarbazoles, azo dyestuffs, aminostilbenes, aminodiphenyls, many other aromatic amines, metals and plastics (to name but the chief)—most of which must operate through initially different biochemical routes. It also became clear that, even were some abnormality in steroid metabolism the underlying cause of spontaneous carcinogenesis, this would not in itself elucidate the fundamental mechanism of action. What unquestionably emerged from the earlier work on the carcinogenic hydrocarbons was a satisfying and indeed brilliant correlation between chemical constitution and biological action, whereby the carcinogenic subclasses of polynuclear hydrocarbons could clearly be related to the parent substance phenanthrene, in a system suggesting dependence upon certain optimal features of molecular size, shape, substitution and reactivity. Yet chemical carcinogenesis should be regarded as a special case of the action of molecules on cells, and Ehrlich's adage applies here as elsewhere: *corpora non agunt nisi fixata*. A growing volume of work is, accordingly, being directed to the nature of the cellular substrates with which the carcinogens combine; and already there is evidence, from several different sources independently, that the combination may lead, either directly or indirectly, to the elimination of certain key proteins or enzyme-proteins essential in the regulation of normal growth, thus liberating more primitive synthetic reactions upon which the process of cell division depends, and from the uncontrolled impetus of which it then proceeds more or less continuously.

The problem of the mechanism of action of the carcinogenic hydrocarbons continues to be attacked from many angles—biological, chemical, biochemical, pharmacological, physical, physico-chemical, and from the aspect of theoretical physics. The early histological effects of various carcinogenic hydrocarbons on mouse skin have been studied by Orr (1955), by Biesele, Grey & Mottram (1956), and by Klinken-Rasmussen (1955) with reference to the hair cycles. Berenblum (1954) has developed his view that the carcinogenic process is divisible into " initiating " and " promoting " stages, and that whereas the former results in a sudden permanent change in the potentiality of the normal cell, the latter, by delaying maturation, allows a sufficient number of undifferentiated daughter cells to accumulate, and thus reach what he conceives as a colony of critical size. From these, and other studies by Roe (1956), Salaman & Roe (1956a), and Gwynn (1957), one gains not only a measure of the value of the general theory, but also an impression that the true situation is perhaps less clear-cut and schematic than was earlier supposed.

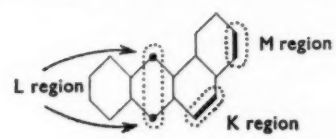
The difficult and thus far unsolved question of the intracellular site of action of the carcinogenic hydrocarbons has been further investigated by Calcutt & Payne (1954), with evidence, however, that the distribution of administered 3:4-benzopyrene within mouse liver cells can be extraordinarily diffuse. Boyland (Boyland & Wiltshire, 1953) has continued his studies of the metabolism of polycyclic compounds, and has also extended them to the reaction with purines and nucleic acids (Booth & Boyland, 1953). In a spectroscopic study of the nature of the complexes of purines with aromatic compounds, Booth, Boyland & Orr (1954) found that the changes in infra-red absorption, on formation of crystalline complexes of caffeine and tetramethyluric acid with polycyclic hydrocarbons, dibenzocarbazoles, and other polycyclic aromatic compounds, were slight but always in the same direction, the results supporting the suggestion that the complexes owe their formation to forces of attraction between the two components arising from their mutual polarization.

The direct interaction of polycyclic hydrocarbons with dermal constituents has been studied by Woodhouse (1955), and on a large scale by the Madison (Wisconsin) school. Wiest & Heidelberger (1953) had shown, following topical application of 1:2-5:6-dibenzanthracene-9,10-^{14}C to the skin of mice, that irreversible binding takes place between the carcinogen or its metabolite and the nucleoproteins, particulate or soluble proteins, but not with nucleic acids. It was then demonstrated by Heidelberger and his co-workers (Hadler & Heidelberger, 1953; Bhargava & Heidelberger, 1954) that no radioactivity is extractable (indicating covalent binding) and that essentially all the radioactive material is combined chemically not only with the original denatured protein but also with the polypeptides derived therefrom. More recently, these studies have been extended (Heidelberger & Davenport, 1956; Heidelberger & Moldenhauer, 1956), with special reference to the dibenzanthracene degradation product 2-phenylphenanthrene-3:2′-dicarboxylic acid (Bhargava, Hadler & Heidelberger, 1955), and to the nature of its bond with protein (Bhargava & Heidelberger, 1956). As so often happens, the existing facts are inadequate, either to provide a final explanation, or to remove certain inconsistencies. Nonetheless, this considerable contribution points to a mechanism whereby proteins, or indeed key enzyme-proteins, either singly or in an interdependent enzyme system, could be deleted from the cell; and it supports a growing picture of biochemical loss in carcinogenesis, which is indeed the main theme of the present review, and for which other evidence will be quoted below. These and other ideas arising from the work of the Madison group have been brought together by Rusch (1956), in what he calls an integrated concept of carcinogenesis.

As part of a general study of the retardation of benzaldehyde oxidation, Dunn, Waters & Roitt (1954) described the

actions in this regard of a number of polycyclic aromatic hydrocarbons. Since carcinogens can act as inhibitors of biological oxidations (cf. Boyland, 1932), presumably by affecting certain types of enzyme oxidase systems (Rusch & Kline, 1941), the possibility of correlating carcinogenic action with the inhibition of the chemical autoxidations of compounds of lipoid type had naturally been envisaged by Rusch and his colleagues. In the contribution by Waters and his school (see also Turner & Waters, 1956), a kinetic study of the retardation of the benzoyl peroxide-catalysed autoxidation of benzaldehyde by anthracene and by 1:2-5:6-dibenzanthracene indicated that chain termination is initially effected by combination of benzoylperoxy radicals with the hydrocarbon. After longer periods of action, polycyclic hydrocarbons of different structure show differences in their type of behaviour, and classification is possible along the following lines: (i) hydrocarbons which retard autoxidation initially but are oxidized to products (quinones) having no such action; (ii) initial inhibitors yielding oxidation products which are also inhibitors; and (iii) very poor inhibitors which are oxidized to more effective inhibitors. It is of much interest that carcinogenic activity appears mainly to be associated with the hydrocarbons of group (ii).

In the endeavour to relate the characteristics of the active centres of the aromatic hydrocarbons to carcinogenic activity, nothing has been more remarkable than the position developed over the past 12 years by the French school of theoretical physics. Otto Schmidt (1938, 1939a, 1939b, 1941) was the first to compare the electronic density of specific regions of certain carcinogenic substances with that in related non-carcinogens, and on the basis of this comparison he postulated as a necessary condition of carcinogenic activity that the density of such regions should exceed 0.44e/Å^2. In particular, he further suggested that the activity of a carcinogen is due to the electro-affinity of its excited state, which facilitates a quantal change in neighbouring molecules. Towards an appreciation of this whole field, a valuable contribution was made by Coulson (1953), of which not the least merit is its comprehensibility not only to the general chemist but in part to the biologist as well. Woernley (1954) regards it as more plausible that the carcinogenic character of phenanthrene derivatives, such as methylcholanthrene, is a result of the over-all electronic configuration of the molecule, rather than that it is attributable to any specific feature such as the 9:10-double bond or so-called K region, although this may be one of a number of important determining factors. Hence, the relationship between the K region and carcinogenicity has doubtless been weighted much too heavily. A marked modification of Pullman's earlier position has, however, been evident in the more recent papers (Pullman, 1953a, 1953b, 1953c, 1953d), where he develops a more complex energetic index, of which a minimum numerical value is requisite for carcinogenicity. According to this view, biological activity is in part determined not only by a favourable K region, but also by an L region (XI; e.g., the *meso* positions of anthracene),

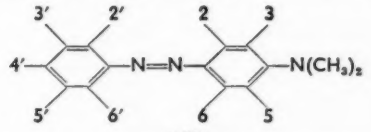


(XI)

not so reactive as to engage the molecule in a different interaction. In general, the K region is selective or permissive, and the L region exclusive. The Pullmans have also endeavoured to apply the correlation between electronic structure and carcinogenic activity to the interpretation of metabolic reactivity (Pullman & Pullman, 1954; Pullman & Baudet, 1954; Pullman, 1954; cf. Daudel & Daudel, 1950), and deduce an activation towards electrophilic attack of the metabolically reactive centre of the molecule (XI; M region), calculations being in agreement with the hypothesis of perhydroxylation. The latest phases of the whole subject are extensively reviewed by Pullman & Pullman (1955a, 1955b; see also Berthier, Berthod & Pullman, 1956; Pullman, 1957; Chalvet & Daudel, 1956; Nagata, Fukui, Yonezawa & Tagashira, 1955).

2. Azo Carcinogens

Since the date of Badger & Lewis's review of the relationship between chemical constitution and biological activity in the carcinogenic azo compounds (Badger & Lewis, 1952), Miller, Miller & Finger (1953) have described the enhancement of activity by fluoro-substitution in 4-dimethylaminoazobenzene (XII), concluding that no position on the prime ring



(XII)

The ring-positions of 4-dimethylaminoazobenzene

is directly concerned in the carcinogenic process. These authors (Miller, Miller & Finger, 1957) also report the requirement for an unsubstituted 2-position. The same school has also carried out extensive studies of the absorption spectra, structure, and relative basicities of the nitrogen atoms, in relation to the carcinogenic activity of aminoazo dyes (Cilento, Miller & Miller, 1955), and of the inability of 4-dimethylaminoazobenzene to act as a major source of labile methyl groups (Miller, MacDonald & Miller, 1955). The carcinogenicity of ethyl derivatives of 4-aminoazobenzene has been studied by Sugiura, Crossley & Kensler (1954) and Sugiura, Kensler & Crossley (1955), a series of ethyl derivatives of this compound and of *N*-methyl-4-aminoazobenzene being tested in rats fed equimolar amounts in a non-protective diet. Introduction of an ethyl group decreased or abolished activity, with the sole exception of *N*-methyl-4′-ethyl-4-aminoazobenzene, which proved of extreme potency in marked contrast with the low activity of *N*-methyl-4′-methyl-4-aminoazobenzene. Carcinogenicity was similarly decreased or abolished by substitution of ethyl groups on the amino group. In a study of the relation of basicity and molecular dimensions to the carcinogenicity of the 4-aminoazobenzenes, Sawicki & Ray (1953, 1954) conclude that a planar configuration seems necessary for carcinogenicity, and that substituents which distort the molecule render it inactive—a situation reminiscent of that which my colleagues and I described for the 4-aminostilbenes. The influence of steric hindrance on the carcinogenicity of 4-dimethylaminoazobenzene has also been discussed by Horner & Müller (1956). Sawicki and Ray further suggest it as probable that carcinogenesis by these azo compounds involves the reaction of a base with an acidic cell constituent.

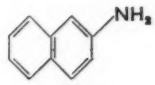
As to the mechanism of action of the azo carcinogens, attention still centres on the hypothesis put forward by the Millers (Miller & Miller, 1953), that the induction of malignant change is attributable to the gradual deletion of key proteins essential for the control of growth—a conception somewhat similar to that which I am gradually approaching from altogether different evidence in another connexion (see section 7). In chemical studies of the protein-bound dyes from rats fed 3′-methyl-4-dimethylaminoazobenzene, Brown, Miller & Miller (1953) describe how crude preparations of the polar dyes, after standing for long periods in solution, gradually liberate small quantities of 3′-methyl-4-monomethylaminoazobenzene, which suggests that the polar dye may be bound to the protein through the amino nitrogen of the dye. Numerous other investigations have been reported—of the structure of the protein-bound derivatives (Rastogi, Miller & Miller, 1956), the factors affecting their formation (Gelboin, Miller & Miller, 1957), the increase of certain soluble liver proteins associated with dye binding (Sorof, Young & Vogt, 1956), the interactions of homologues of the carcinogenic azo dyes with various proteins (Burkhard & Moore, 1955; Burkhard, Bauer & Grossman, 1957), the reactions of ^{14}C-labelled carcinogenic azo dyes with rat-liver proteins (Hultin, 1957), and the distribution of the protein-bound azo dye in different cellular fractions (Hultin, 1956a, 1956b).

The deletion hypothesis must not of course be accepted too uncritically, and like every other it is still *sub judice*, certain facts being in its favour, but not necessarily all. It was believed to receive a certain degree of support from independent studies, such as those of Weiler (1952, 1954, 1956a) on the serological organ-specificity of the liver, using a fluorescence method of antigen detection in the course of carcinogenesis with 4-dimethylaminoazobenzene, whereby it appeared that the process was accompanied by the gradual deletion of a liver antigen. In attempts to confirm the experiments of Weiler—undertaken because of the support which the latter also seemed to give to the thesis of Burnet (1957) that the control of cell multiplication may in part be mediated by " self-marker " antigens, and to Green's immunological hypothesis of carcinogenesis (Green, 1954)[1]—Hughes, Louis, Dineen & Spector (1957) succeeded in reproducing the appearances which Weiler had observed in sections of livers from rats fed on azo dye, but were unable to agree with his interpretation, that the loss of organ-specificity is responsible. Nevertheless, these authors admit that their results in no way invalidate the concept that the malignant cell has lost the self-marker recognition units necessary for the control of multiplication; and admit that the appearances observed by Weiler may well be an expression of deletion or alteration of the highly specific dye-binding proteins. There the matter must rest for the moment. In spite of criticism, deficiencies and certain inconsistencies, the deletion hypothesis still has much in its favour. As has been seen, it derives a certain support from studies not only of the azo carcinogens but also of the carcinogenic hydrocarbons; and similar general support is afforded in the field of the alkylating carcinogens, still to be considered. If the hypothesis survives the intensive scrutiny which it deserves and will doubtless receive, an important task will be to define the nature and functions of the affected protein components, in particular to decide which enzymic activities, if any, have been damaged or eliminated in the process.

[1] See also Green, p. 101 of this number of the Bulletin.—Ed.

3. Aromatic Amines

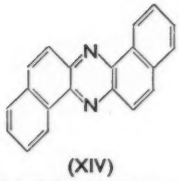
The manufacture and use of certain dyestuff intermediates still present a hazard in the chemical industry[2], in the form of an increased incidence of tumours of the urinary bladder; and Case, Hosker, McDonald & Pearson (1954) have studied the influence of contact with benzidine, 1-naphthylamine, and 2-naphthylamine (XIII), in causing many more bladder



(XIII)
2-Naphthylamine

tumours in workmen so exposed than would appear if no special risk was operating. Furthermore, both the onset of, and death from, these occupational tumours take place at a much earlier age than in non-occupational cases. There is no evidence that aniline causes any increase of bladder tumours in men who handle or manufacture it, but some which incriminates the manufacture of magenta and auramine (Case & Pearson, 1954). 2-Naphthylamine is shown to have been the most potent cause of occupational bladder tumours in Britain between 1915 and 1951, the ratios of the activities of 2-naphthylamine, mixed exposures, benzidine and 1-naphthylamine being 5.2 : 2.7 : 1.7 : 1.0. Tumours appear after an average induction period of some 16 years for 2-naphthylamine and benzidine, and of 22 years for 1-naphthylamine, the average induction time not being appreciably altered by the severity or duration of exposure, and hence appearing to be a feature of the causal agent.

The metabolism of 2-naphthylamine in the rat has been studied by Henson, Somerville, Farquharson & Goldblatt (1954), using the compound 2-[8-^{14}C]-naphthylamine. Excretion of the radioactive compound or its metabolites into the urine or through the bile into the gut, from which they could be re-absorbed, was readily demonstrated. Excretion of 90–95% of the radioactivity of a single dose in the urine and faeces occurs in about three days, but thereafter excretion is slow, and the compound or its metabolites remain detectable for many weeks (see also Somerville, Henson, Cooke, Farquharson & Goldblatt, 1956; Twombly, Zomzely & Meislich, 1957). The carcinogenic activity of 2-naphthylamine has been further studied by Bonser, Clayson, Jull & Pyrah (1956), while Rudali, Chalvet & Winternitz (1955) have tested the possibility that various phenazines, occurring as impurities with the naphthylamines, may be the cause of industrial bladder tumours: phenazine, 1:2-3:4-dibenzophenazine, and 1:2-5:6-dibenzophenazine (XIV), all caused tumours when introduced into the bladders of young Wistar rats.

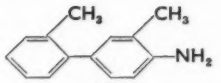


(XIV)
1:2-5:6-Dibenzophenazine

[2] See also papers by Walpole & Williams (p. 141) and by Goldblatt (p. 136) in this number of the Bulletin.—Ed.

In an endeavour to decipher the mode of action of carcinogenic amines generally, Clayson (1953)[3] has suggested that the process is dependent upon conversion to *o*-hydroxyamines (*o*-aminophenols), a conversion which is facilitated if the position *para* to the aromatic amino group is blocked to biological hydroxylation. However, much further investigation is required, and Elson (1952; see also Elson's paper on p. 161 of this symposium, and Elson, Goulden & Warren, 1958) in particular has pointed out that, while it is certainly possible that *o*-aminophenols are potentially carcinogenic, other factors —for example excretion as ethereal sulphates rather than as glucuronides (in which form are excreted the carcinogens benzidine, 4-aminodiphenyl and 4-aminostilbene)—may greatly modify their action. The carcinogenic properties of *o*-aminophenols and related compounds have been studied by Bonser, Bradshaw, Clayson & Jull (1956) by bladder implantation, which technique, as also that of intravesical injection, has been of much value in all these studies (Bonser, Crabbe, Jull & Pyrah, 1954; Bonser & Jull, 1956). A remarkable development in investigations of the pathogenesis of bladder tumours is seen in Boyland's implication of 3-hydroxyanthranilic acid as a carcinogen produced by endogenous metabolism (Boyland & Watson, 1956), and in his account of the metabolism of tryptophan in cancer of the bladder in man (Boyland & Williams, 1956). Boyland[4] has also continued his studies of the biochemistry of the aromatic amines as, for example, the conversion of arylamines into arylsulphamic acids and arylamine-*N*-glucosiduronic acids (Boyland, Manson & Orr, 1957), and the process of enzymic hydroxylation by rat-liver microsomes (Booth & Boyland, 1957).

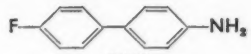
Following their description of the carcinogenicity of 4-aminodiphenyl (xenylamine) and the even more potent 3:2′-dimethyl-4-aminodiphenyl (XV), Walpole, Williams &



(XV)

3:2′-Dimethyl-4-aminodiphenyl

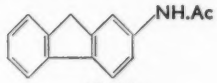
Roberts (1952) suggested that the former might be capable of causing bladder tumours in man, the presence of this base in the distillation residues of " heavy aniline ", together with naphthylamines, having been suspected as partly responsible for early " aniline cancers ". In fact, Melick and his coworkers in 1955 (Melick, Escue, Naryka, Mezera & Wheeler, 1955) described the first reported cases of human bladder tumours due to the new carcinogen xenylamine. Walpole, Williams & Roberts had earlier (in 1954) observed tumours of the urinary bladder in dogs, produced by feeding this substance, which they described as a more effective carcinogen in this species than either benzidine or 2-acetamidofluorene, and at least as potent as 2-naphthylamine. Walpole, Williams & Roberts (1955) also reported bladder tumours in rats of two strains, induced with 3:2′-dimethyl-4-aminodiphenyl. Hyperplastic nodules in rat mammary glands, following the feeding of 4-acetamidodiphenyl, had been reported by Tuba, Rawlinson, Fraser & Jeske (1953); more recent papers deal with this and other aspects of the carcinogenicity of aminodiphenyl and its derivatives (Miller, Sandin, Miller & Rusch, 1956; Deichmann, Coplan, Woods, Anderson, Heslin & Radomski, 1956; Morris, Velat & Wagner, 1957). Such compounds as 2:2′-diaminodiphenyl, 2-amino- and 4-aminodiphenylamine have been reported in the products of benzidine rearrangement (Večeřa, Petránek & Gasparič, 1956). Hendry, Matthews, Walpole & Williams (1955) made a fresh observation of much interest, namely the capacity of the fluorine derivative (XVI) to induce adenoma and adenocarcinoma of the



(XVI)

4-Amino-4′-fluorodiphenyl

kidney in the rat: the corresponding chloro-compound is also active, but the bromo-derivative is not (Walpole & Williams, 1958). Numerous other papers describe different aspects of the carcinogenicity of 2-acetamidofluorene (XVII) (Morris,



(XVII)

2-Acetamidofluorene

1955) and aminofluorene (Schinz, Fritz-Niggli, Campbell & Schmid, 1955), the involvement of the former in *o*-hydroxylation and in protein-binding (Weisburger & Weisburger, 1954; Peters & Gutmann, 1956; Dyer & Morris, 1956), and the patterns of its metabolism and excretion (Weisburger, Weisburger & Morris, 1954, 1956; Weisburger & Weisburger, 1955; Weisburger, Weisburger, Morris & Sober, 1956; Urquhart, 1955; Dyer, 1955); also the fate of the methyl group in *N*-methyl-[^{14}C]-2-aminofluorene, and the carcinogenicity of *N*-methyl- and *NN*-dimethyl-2-aminofluorene (Weisburger, Weisburger, Velat & Morris, 1956), the chemistry of *N*-substituted 1-, 2- and 4-aminofluorenes (Sawicki & Chastain, 1956), and the colour reactions of the carcinogenic aminofluorenes with montmorillonite (Pinck, 1956). Finally, the quantitative aspects of the carcinogenicity of 4-dimethylaminostilbene have been investigated (Druckrey & Schmähl, 1956), and the production of stomach carcinoma in the rat by 4-nitrostilbene described (Druckrey, Schmähl & Mecke, 1955). Measurements of the interaction of proteins and aminostilbenes have been reported by Cheesman (1955). All these studies testify to the striking affinities, on the grounds both of their chemical properties and their biological effects, between the aminodiphenyls, aminostilbenes and aminofluorenes; although in no case can it be claimed that anything is known, at least with precision, of their proper mode of action.

4. Polymers and Plastics

Under this heading can be included a range of macromolecular agents—some, however, so unreactive that they may operate rather by physical means—and if the mechanism of their action is in all cases obscure, they serve at least to prove that the pathway to malignant transformation can be entered from many different channels.

An unusual observation was made when Turner (1941) accidentally found that bakelite discs implanted for long periods in albino rats eventually evoked fibrosarcomata. The observation remained isolated for some years until Oppenheimer, Oppenheimer & Stout (1948a, 1948b), in the course of experiments performed on rats to produce hypertension by

[3] See also Bonser, Clayson & Jull, p. 146 of this number of the Bulletin.—Ed.
[4] See also Boyland, p. 153 of this number of the Bulletin.—Ed.

wrapping regenerated cellulose fibre (cellophan) around one kidney, again quite unexpectedly recorded sarcoma development in the neighbourhood of the cellophan. The same result was later found to follow subcutaneous implantation in the abdominal wall and, pursuing the observation, these authors (Oppenheimer, Oppenheimer & Stout, 1952) were successful in producing sarcomata in rats by embedding various plastic films—of alcohol-extracted cellophan, commercial polyethylene, pure polyethylene, and polyvinyl chloride—in the subcutaneous tissues. Sarcomata were also produced in mice, although in a smaller proportion. In later papers, the Oppenheimers have given an account of this fascinating observation and their contributions towards it, whereby it has been established that sarcoma can be induced by implantation into the tissues of rodents not only of the above, but also of Dacron film, Kel-F, Nylon, polystyrene, Silastic (a commercial silicone film), and Teflon film (Oppenheimer, Oppenheimer & Stout, 1954; Oppenheimer, Oppenheimer, Danishefsky, Stout & Eirich, 1955). It is of interest that in certain experiments, whereas removal of the plastic films up to about six months after implantation obviated tumour development, tumours appeared in spite of removal of the films after this period (personal communication). In control experiments, only foreign body reactions were obtained with cotton fibres or linters as used in the manufacture of cellophan, or with surgical cotton. One tumour appeared in 50 rats implanted with chemically clean cover-glasses, and Druckrey & Schmähl (1954a) have described tumours induced by the implantation of quartz. Other related observations include the production of sarcomata by methyl methacrylate implants (Laskin, Robinson & Weinmann, 1954), and a confirmation of the production of tumours in rats by the implantation of pure polyethylene (Bering, McLaurin, Lloyd & Ingraham, 1955). Bing (1955) has studied the tissue reaction to implanted plastics, and Nothdurft (1955) the dependence on shape and size of the induction of sarcomata by implantation of foreign bodies.

Oppenheimer (personal communication) had also obtained a single fibrosarcoma 313 days after embedding silk film in the tissues of an albino rat, perhaps the first instance in which a tumour may have been induced by a natural polymer. This kind of observation, and the general inertness of such polymers as polyethylene, Terylene, and Teflon, make any purely "chemical" theory of action somewhat unlikely, although physical factors by themselves equally seem inadequate to provide the whole explanation. Druckrey & Schmähl (1952) had postulated residual valencies on the polymer surfaces, thus enabling the latter to bind linear cell proteins, but were compelled to review this interpretation on confirming the carcinogenic action of polyethylene (Druckrey & Schmähl, 1954b). However, they quote Hopff as showing that polyethylene can become cross-linked by oxygen, so that it may not, therefore, be as inert as is commonly supposed; mention is also made of the suggestion of Eirich, which could not however apply in the case of the polyamides prepared by condensation, that free radicals may be trapped during polymerization, and so be responsible for the biological action. Doubtless the explanation must await further investigation, perhaps along the lines of Danishefsky's experiments (personal communication), using radioactive polymers, such as polymethylmethacrylate, and polystyrene, labelled on the methanolic and β carbons respectively. However, polytetrafluoroethylene films have also been found by Oppenheimer to be carcinogenic; these are completely inert, unlike polyethylene do not react with oxygen, and are in fact so chemically resistant that it is difficult to envisage any reaction into which they can enter. One possibility, being investigated at the moment, is whether the polymer or plastic films do not simply induce a state of local anoxia or anaerobiosis—recalling the experiments of Goldblatt & Cameron (1953), which were inspired by the views of Warburg, and in which malignant change in vitro appeared to be achieved by intermittent deprivation of air, and the substitution of nitrogen, during prolonged propagation.

5. Miscellaneous Chemical Agents

Apart from all the above, there is a great range of endocrine, dietary and toxic agents or influences capable of inducing malignant change. The first two classes are perhaps strictly outside the purpose of this paper, yet we may mention the appearance of squamous-cell carcinoma of the skin in rats after prolonged treatment with thiourea (Rosin & Rachmilewitz, 1954), a thiourea-induced myxomatous tumour of the rat (Rosin, 1956), and the induction of malignant tumours in the eyelids and the auricular region of thiourea-treated rats (Rosin & Ungar, 1957). Other examples of the extreme and indeed bizarre varieties of chemical agents capable of evoking malignant change include the carcinogenic effect of the sulphonamides (Bjerre Hansen & Bichel, 1952), tumour production by phenol and related compounds (Boutwell, Rusch & Booth, 1956), the carcinogenic action of 4-nitroquinoline-*N*-oxide (Nakahara, Fukuoka & Sugimura, 1957), and of parafuchsin (Druckrey, Nieper & Lo, 1956), the induction of fibrosarcomata by subcutaneous injection of carboxymethylcellulose, polyvinylpyrrolidone and polyoxyethylene sorbitan monostearate (Tween 60) (Lusky & Nelson, 1957; Hueper, 1957), and the tumour-promoting and cocarcinogenic effects of various non-ionic lipophilic-hydrophilic surface-active agents (Setälä, 1956).

As an outcome of their earlier work on the carcinogenic action of tannic acid, Korpássy and his co-workers have described the influence of dietary protein (Korpássy & Mosonyi, 1953), the production in rats of a haemoblastosis with this agent (Korpássy, Sztanojevits & Koltay, 1954), and the rapid production of malignant hepatomata by the simultaneous administration of tannic acid and 2-acetamidofluorene (Mosonyi & Korpássy, 1953). This work is of much interest in relation to the cross-linking mechanism suggested for the aromatic nitrogen mustards (see below), and it is reinforced by recent and unpublished experiments by K. S. Kirby, in which sarcomata in mice have resulted from the subcutaneous injection of various vegetable tannins.

Many contributions have confirmed the specific carcinogenic action of urethane upon the mouse lung, and have explored the nature of its action (Baló, Juhász & Varga, 1953; Rogers, 1954a). The fasting of mice for a day before and after injection of urethane was found greatly to increase the number of tumours resulting, an effect prevented by crude casein but not, however, by methionine, which, indeed, appeared further to increase the tumour yield (Rogers, 1953a). According to Malmgren & Saxén (1953), the carcinogenic effect of urethane is largely confined to the first day after injection and is absent on transplanted lung tissue 24 hours after administration of urethane to the host. There persists, however, some factor which enhances the tumour incidence

in urethane-treated lung tissue, transplanted into hosts previously treated with urethane, over the incidence in the same tissue transplanted into untreated animals. Rogers (1953b) has studied the effect upon the initiation of pulmonary adenomata in mice with urethane, of substances known to influence nucleic acid synthesis; mice given sodium deoxyribonucleate intraperitoneally a day before and at the time of subcutaneous injection of urethane were found to develop strikingly fewer tumours than did controls, while, contrariwise, prior treatment with aminopterin increased the tumour yield. In an investigation of the effect of known precursors of nucleic acid pyrimidines (Rogers, 1954b), orotic acid reduced the number of adenomata produced, and other results suggested that the action of urethane might take place through its conjugation with another substance, possibly oxaloacetic acid or a related compound, the conjugate interfering competitively with pyrimidine synthesis. Apart from its action in evoking pulmonary adenomata, Salaman & Roe (1953) have described urethane as an initiator (in Berenblum's specific sense) of the formation of tumours of the skin in mice.

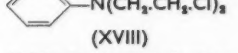
Last to be considered is the field of inorganic carcinogens. As was pointed out by Hueper (1950), arsenicals were for more than a century the only metallic substances showing, upon occupational, environmental, or medicinal exposure, a causal relationship to cancer in man. Later observations in occupational carcinogenesis have, however, incriminated many metals or metallic compounds—with varying degrees of certainty—such as chromates, nickel carbonyl and asbestos, while, experimentally, beryllium, selenium and nickel (Hueper, 1952) have been proved carcinogenic for certain species. In the case of nickel, Hueper (1952; see also Hueper, 1955a) injected the pure metallic powder into the femur, pleura and nasal sinus of rats, and observed tumours (osteogenic sarcomata and tumours arising from the connective tissue and abdominal lymph nodes), in 30% of animals dying after 7–16 months. The production of bone sarcoma in rabbits by the intravenous injection of beryllium compounds was described by Gardner & Heslington (1946; see also Nash, 1950a, 1950b; Barnes, 1950), and Cloudman, Vining, Barkulis & Nickson (1949), Dutra & Largent (1950), Dutra, Largent & Roth (1951), Hoagland, Grier & Hood (1950), Sissons (1950) and Janes, Higgins & Herrick (1954) obtained similar results. Hueper (1955b) has also studied the tissue reactions to parenterally introduced powdered metallic chromium and chromite ore, and Grogan & Oppenheimer (1955; see also Grogan, 1957) studied the interaction of chromium III and chromium VI compounds with proteins. Sarcomata in rats, following the injection of cobalt powder, and the effect of cobalt on mitosis in tissue culture, have been described by Heath (1954a, 1954b, 1957), while the general question of the carcinogenic effect of metals in rodents has been dealt with by Oppenheimer, Oppenheimer, Danishefsky & Stout (1956).

The above examples (which are by no means inclusive) illustrate the wide range of chemical agents capable of inducing a carcinogenic response. It is one of our main tasks to decide whether the ultimate mechanism is fundamentally the same in all these cases, in spite of the variety of initial causes by which the end-result can be evoked.

6. Alkylating Carcinogens and Mutagens

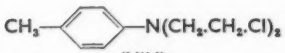
It is remarkable, in view of the great effort directed towards it, that so little is in fact known of the mechanism of action of the cyclic hydrocarbons, the azo dyestuffs, the aromatic amines, and other carcinogens, or of the precise site in the cell at which such action takes place. Over the past ten years there have, however, been obtained the first hints as to the likely mode of action of at least one class of chemical carcinogen, from the discovery of carcinogenic activity in a series of nitrogen mustards and (later) of other agents—the diepoxides, polyethyleneimines and dimethanesulphonyloxyalkanes—all of which are capable of alkylating various cell components and receptors (Haddow, 1949, 1950, 1951). In this case, observation of the carcinogenic properties of such compounds came long after investigation of the so-called mustards as potential agents of chemical warfare (cf. Winternitz, 1920). The discovery of carcinogenic activity in this new chemical class, in substances of relatively high reactivity, simple in structure, and yet possessing molecular features strongly suggestive, if not, indeed, indicative, of their mechanism, has led to much progress and to a theory of action which has in turn stimulated many other developments. Whether the suggested mechanism is at all applicable to other carcinogens remains, of course, an open question.

It appears that the main primary action of the nitrogen mustards upon dividing cells is highly direct, that it is exerted mainly during the interphase between divisions, that the resulting damage becomes cytologically manifest only during mitosis, and that such damage may accumulate in successive divisions, until the cell either becomes no longer viable, or undergoes malignant change. Many of the biological effects which these substances are capable of producing, and the cytological changes which form their basis, fully justify the description of such agents as " radiomimetic ", even though there are significant differences between them and ionizing radiations in the details of their action. This biological parallelism between ionizing radiations and the nitrogen mustards has led to attempts to relate their action quantitatively, through the concept of the radioequivalence of radiomimetic chemical agents in their effects on cells both in vitro and in vivo, and in their reaction with possible biological receptors such as deoxyribonucleic acid. It was also the radiomimetic quality of the mustards in general which raised the question whether these agents might not also prove to be carcinogenic. This possibility was tested with abundantly positive result, since pronounced carcinogenic activity (mainly after subcutaneous injection in the mouse, rat and hamster, but also following feeding) was shown by several aromatic derivatives (e.g. XVIII–XXII). Independently, Boyland &



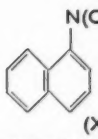
(XVIII)

NN-Di-2-chloroethylaniline



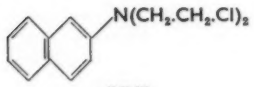
(XIX)

NN-Di-2-chloroethyl-*p*-toluidine



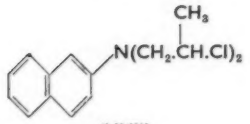
(XX)

1-Naphthyl-di-2′-chloroethylamine



(XXI)

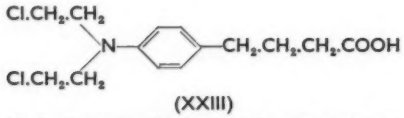
2-Naphthyl-di-2′-chloroethylamine



(XXII)

2-Naphthyl-di-2′-chloro-*n*-propylamine

Horning (1949) had also observed tumours (mainly of the lung) in mice receiving the aliphatic compounds HN2 (*NN*-di-(2-chloroethyl)-methylamine) and HN3 (tri-(2-chloroethyl)-amine), and Heston (1949) had described the induction of pulmonary tumours in Strain A mice treated with HN2, or (Heston, 1953a; see also Heston, 1953b; Heston, Lorenz & Deringer, 1953) exposed to mustard gas. In further confirmation, Griffin, Brandt & Tatum (1950, 1951) observed adenocarcinoma of the lung, leukaemia, lymphosarcoma, angiosarcoma, and osteogenic sarcoma, in rats and mice given HN2 and HN3 by the intravenous, intraperitoneal, and subcutaneous routes, in some cases after a single dose. More recently, Rogers (1955) has studied the induction of pulmonary adenomata in mouse lung tissue with nitrogen mustard in vitro, and Salaman & Roe (1956b) have described *NN*-di-2-chloroethyl-*p*-aminophenylbutyric acid (XXIII) as an initiator of skin tumour formation in the mouse.



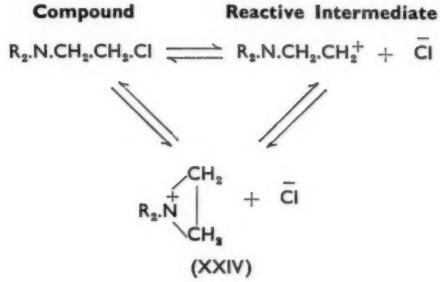
(XXIII)

NN-Di-2-chloroethyl-*p*-aminophenylbutyric acid

At an early stage in the investigations by my colleagues and myself, it became obvious that the tumours induced by the aromatic nitrogen mustards showed an exceptional incidence of nuclear abnormalities, similar in character to the well-recognized abnormalities produced by the same compounds in dividing cells. Nuclear abnormalities may also be encountered in tumours induced by the cyclic hydrocarbons, and these observations recall the nuclear imbalance frequently seen in tumours generally, and once more direct attention to the nucleus as the possible primary site of carcinogenic action. At the same time, such gross changes are probably not causal, but merely associated events, and the essential alteration underlying malignancy is almost certainly much more specific and elusive.

For perhaps the first time in the study of carcinogenesis, the mustards provided agents with characteristic molecular features immediately suggestive of possible mechanisms of action. In efforts to establish the requirements for optimal activity, and to correlate such requirements with chemical reactivity, it became clear that biological activity is associated with the haloalkyl chains exclusively, of which, in both the aliphatic and aromatic series, a minimum of two is required (Haddow, Kon & Ross, 1948). Seeking the explanation for this bifunctional or polyfunctional requirement, Goldacre (considering reversible denaturation and the folding and unfolding of chromosome chains in relation to cell division, and the possible adsorption of carcinogenic molecules to the unfolded state), Loveless (from the aspect of chemical cytology), and Ross (from a study of the kinetics of reaction of such two-armed compounds), suggested that the two groups might be required to permit the molecule to react at two distinct points lying either on a single surface or fibre, or on two contiguous fibres (Goldacre, Loveless & Ross, 1949). Of the various possibilities, cross-linkage between the constituent macromolecular fibres of the chromosome itself was of particular interest, in the light of the growing assumption that reaction of the mustard carcinogens might well be directly with genetic material. The latter had already been postulated in respect of chemical mutagenesis (Auerbach & Robson, 1947), while the cell was known to be susceptible to the effects of the mustards at a time when there exists within it a system of parallel identical fibres, the gene strings of the sister chromatids. Nevertheless, the cross-linkage hypothesis, which was first suggested as perhaps the most valid interpretation of the available facts, is now known to be unduly simple. Other possibilities are perhaps equally likely, as for example combination not with a genetic determiner directly, but with a precursor or substrate; the anchoring of one arm followed by attachment of the second to some less accessible site on the same fibre; polymerization of the agent within the cell; a more random ramification or internal polymerization, preventing the movement of molecular chains; and, finally, the blanketing of receptors by a high concentration of monofunctional analogues. Also, the hypothesis is refractory to test, in view of the difficulty in defining reliable criteria for cross-linking even in model systems, the fact that demonstration of cross-linking capacity in a model system provides no proof of such a mechanism within the cell, and conversely that cross-linking is not excluded through inability to demonstrate it in some artificial system. In actual experience, relatively little direct evidence of cross-linkage has been obtained, although recently Alexander (personal communication) has been able to demonstrate the process occurring via the phosphate groups in the nucleoprotein of herring sperm. There is however no doubt of the powerful stimulus which the brief paper by Goldacre *et al.* (1949) exerted, or of the value of the new findings which came about as the result.

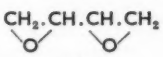
The studies by Ross of the reactions of the haloalkylamines in aqueous solutions under mild conditions have shown that these compounds should be regarded as alkylating agents (Ross, 1953). When such compounds enter into chemical reactions, these can be interpreted on the assumption of the formation of a carbonium ion as the reactive intermediate (XXIV). A carbonium ion is electrophilic and will react with



(XXIV)

any nucleophilic centre, the main such centres in biological systems being organic and inorganic anions and bases. However, whether a particular group is in a form capable of reacting at physiological pH will depend upon its dissociation constant. On this consideration, the groups most likely to react with the alkylating agents are the carboxyl group and the terminal α-amino and histidine amino groups in proteins, and the phosphoryl and so-called aromatic type amino groups of the nucleic acids. Ross is of opinion that the more nearly do the conditions approach those in living tissues, the more significant becomes the reaction with carboxyl groups. But another attractive possibility is that the acid groups in the biological reaction are those in nucleoproteins, and Butler, Gilbert, James & Ross (1951) have for example suggested that the loss in structural viscosity of deoxyribonucleic acid preparations, after treatment with mustards, might be connected with the formation of unstable tri-substituted phosphoric esters by reaction of the primary phosphoryl groups in the nucleic acid. Also, Davis & Ross (1952) indicate that linkages which are more stable may be formed by the reaction of chloroethylamines with such phosphoryl groups in nucleic acids, than are formed with carboxylic groups in proteins.

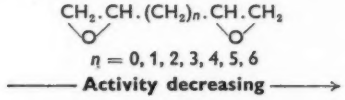
The characteristic feature of the radiomimetic mustards is their ability to act as bifunctional electrophilic reagents under mild conditions in aqueous solutions (Davis, Everett & Ross, 1950). Since epoxides also were known to act as electrophilic reagents under the same conditions (Hammett, 1940), and to react readily with proteins at physiological pH (Fraenkel-Conrat, 1944), Ross (1950) was led to include them for investigation. Before they could be examined, however, the same suggestion was made to me by J. B. Speakman, as an immediate outcome of the cross-linking hypothesis, and because of the application of various diepoxides as cross-linking agents in textile practice (Speakman, 1948). It appeared that the simplest member of the series, namely 1:2-3:4-diepoxybutane (XXV) had already been investigated in textile research



(XXV)

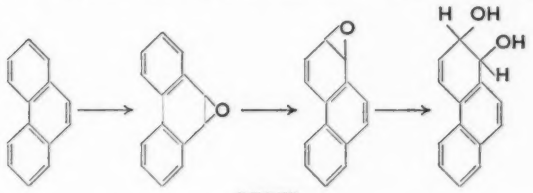
1:2-3:4-Diepoxybutane

in Germany, in the treatment of wool (Alexander & Whewell, 1946), and cross-linking by diepoxides was later studied by Deuel (1947). No sooner had the above suggestion been put to the test than it became obvious that a series of diepoxides (Everett & Kon, 1950) was indeed capable of producing biological effects largely indistinguishable from those due to the radiomimetic mustards, activity decreasing in the manner shown in (XXVI). Very shortly, the parent substance 1:2-3:4-diepoxybutane was also shown to be carcinogenic.



(XXVI)

Epoxides occur among the products of the autoxidation of unsaturated fatty acids, and it is possible they may play a significant part in various biological oxidations. Earlier consideration of the metabolic transformation of the carcinogenic hydrocarbons had included the possible importance of hitherto unrecognized metabolites such as epoxides, and in this connexion Boyland & Wolf (1950) had unsuccessfully attempted the synthesis of phenanthrene epoxide. Very naturally, discovery of carcinogenic action in the diepoxides again drew attention to this point. The oxygen atom of an epoxide group is capable of migration and, although in this case the reaction may perhaps appear improbable, Boyland suggested that if carcinogenic hydrocarbons react with oxygen first in the K region (see above), the oxygen may then migrate to the adjacent double bond in the side ring (see XXVII).



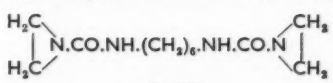
(XXVII)

Hypothetical stages in the metabolism of phenanthrene

The hypothesis of epoxide formation by the carcinogenic hydrocarbons is as yet supported by little evidence, but it would help to explain why the superficially unreactive polycyclic hydrocarbons produce such profound biological effects, and would certainly effect an astonishing linkage between the action of carcinogens of apparently very diverse type.

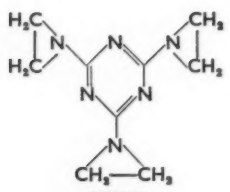
Completely independent of the above, intensive investigation of the chemistry of the diepoxides in the laboratories of Canadian Industries Limited, Montreal, together with a consideration of the cross-linkage hypothesis, led Hendry, Homer, Rose & Walpole (1951a) to a study of the cytotoxic activity of the diepoxides, with special reference to a suggestion by Rose that these substances might produce their effects not as individual molecules but rather as multi-reacting polymers. While Rose's concept of polymer formation would assuredly prevent combination of nucleic acid with histone, and could readily cause dissociation of nucleoproteins, a polymerized structure would not on the other hand appear necessary to produce such blocking effects, in evidence of which may be quoted the undoubted activity of certain monofunctional imines (see below), and of certain monofunctional mustard and other compounds in sufficiently high concentration in vitro.

The application of bifunctional and polyfunctional agents to the treatment of rayon received particular attention by the I.G. Hoechst Farbwerke and other groups in Germany during the Second World War (see Hill, 1946; Evans, 1949), and specially prominent among substances found to react with cellulose were the compounds hexamethylenediamine diethyleneurea (XXVIII) and triethyleneimino-1:3:5-triazine (triethylenemelamine (TEM)) (XXIX)—representatives of a large class of polyethyleneimines with cross-linking ability and the capacity to effect a great reduction in the swelling properties of both artificial and natural fibres. While imines had earlier been considered in related technical applications (e.g. British Patent Specification 472.899, 1937), these later



(XXVIII)

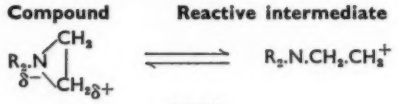
Hexamethylenediamine diethyleneurea



(XXIX)

2:4:6-Triethyleneimino-1:3:5-triazine

German developments, and the chemical work of Bestian (1950), unquestionably played a large part in directing the attention of several groups of workers independently to them, as cross-linking agents with a reactivity towards nucleophilic reagents similar to that of the mustards and epoxides (XXX),

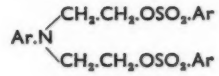


(XXX)

and likely to manifest similar cytotoxic and carcinogenic action. This surmise proved correct and, as for all the other examples so far considered, compounds possessing at least two reactive groups, in this case the ethyleneimine ring, showed greatest cytotoxic activity. Hendry, Homer, Rose & Walpole (1951b) found the biological activity of mono-ethyleneimines to be comparatively low, being no more than one-tenth to one-hundredth that of the nearest related *bis*-ethyleneimine derivatives. However, a striking example of biological activity in monofunctional imines is their description of carcinogenicity in stearoyl ethyleneimine ($C_{17}H_{35}CO.N(CH_2)_2$), and in myristoyl and caproyl ethyleneimine: in this series, activity appears to be eliminated by branching of the chain. Later, Walpole, Roberts, Rose, Hendry & Homer (1954; see also Walpole, 1953) described the carcinogenicity of a series of monofunctional ethyleneimine derivatives.

Although clearly it cannot be exclusive, Hendry and his colleagues developed their theory of intracellular polymer formation for the case of the imines as for the epoxides, and extended these notions in a highly imaginative and ingenious way to the carcinogenic hydrocarbons, azo compounds, and aminostilbenes. In the first case, two characteristics were regarded as significant, namely the need for a planar structure of optimal molecular dimensions, and a degree of chemical reactivity sufficient to allow conjugation with protein. (It should be noted that the same considerations apply to the azo carcinogens and aminostilbenes equally.) The synthesis was then visualized as taking place within the cell of a protein or nucleoprotein unit carrying the hydrocarbon moieties as side-chains. The latter, by reason of their lipophilic nature, might then associate in micelles, with the component flat molecules held together in lamellar form. The arrangement would resemble that provided by the reactive polymers derived from the epoxides and ethyleneimines. In essence, the carcinogenicity of the polycyclic hydrocarbons is regarded as a function of requisite chemical reactivity, together with the capacity of the molecules to pack into micellar units. Hendry and his colleagues also drew attention to the suggestion of Mueller & Miller (1950), that the linkage of the carcinogenic azo dyestuffs to protein may be through an intermediate methylol-aminoazobenzene. Since the *N*-methylol group is also capable of conferring cytotoxic activity, this, together with the occurrence of the azobenzene structure in a number of typical micelle-forming dyestuffs (Vickerstaff, 1950), might point to a mode of action whereby the attachment of the azobenzene residues to protein is followed by an orientation into micelles analogous to those proposed for the polycyclic hydrocarbons. Similar arguments could be advanced in the case of the carcinogenic aminostilbenes, the stilbene structure again being associated in dyestuff technology with special colloidal properties.

Apart altogether from the biological examination of cross-linking agents already known from their applications in textile technology, search was also made for new types of bifunctional agent with cytostatic properties. Although most of these efforts were fruitless, biological activity was nevertheless detected in various members of the series (XXXI) (Haddow &



(XXXI)

Timmis, 1951), a result which led to the investigation of other structures bearing sulphonic acid ester groups, and in particular the α : ω-*bis*(methanesulphonyloxy)alkanes (XXXII)

$n = 2$–10

(XXXII)

α : ω-*bis*(Methanesulphonyloxy)alkanes

(Timmis, 1951; Haddow & Timmis, 1953). While all members of this latter series exhibit some activity where $n = 2$–10, the degree of such activity varies considerably from one member to another, in a regular manner, being maximal where $n = 4$ or 5, less where $n = 6$, 7, or 8, and very greatly reduced where $n = 2$, 3, 9 or 10. Again the series is capable of acting through the carbonium ion mechanism, although Timmis suggests that activity may be determined by the ability of these agents to form ring compounds with an amino or sulphydryl group, variations in activity being attributed to variations in the stability of the ring or the rate of its formation. The spatial configurations required to form such a ring, or to esterify two adjacent phosphoryl groups, may however be similar, and the observed relationships could in fact lend support to either hypothesis. However, the activity of this series jeopardizes Rose's theory of the formation of reactive polymers, at least as a general mechanism, since it is impossible to derive such polymers in the dimesyl series. The correlation between tumour induction on the one hand and growth-inhibitory activity on the other is further shown by the carcinogenicity of members of both the above series, 1:4-dimethanesulphonyloxybutane being outstandingly active in this respect. Tumour initiation in mouse skin by various esters of methanesulphonic acid has also been described by Roe (1957).

If it be conceded that a general principle of action is gradually being approached, it is equally evident that the field is still entirely open in the question of biochemical detail. Two examples can be quoted from recent work. First, Roberts & Warwick (1957) have reported the formation of *S*-ethylcysteine from ethyl methanesulphonate in vivo. This observation has served to redirect attention to the views of Louis Rapkine and Mazia—somewhat neglected of later years—on

the relationship of sulphydryl groups to growth and cell division (see Mazia, 1954; Stern, 1956). Secondly, Lawley (1957) has established a mechanism whereby alkylation of the guanine moieties of deoxyribonucleic acid could cause not only extensive changes in physico-chemical characteristics, but also elimination of the alkylated guanines from the macromolecule at physiological pH, through a process which might be expected to facilitate fission of the polymer chain.

7. Interpretation and Conclusions

Whatever the precise chemical mechanism of the action of carcinogens, there can be little doubt of the importance of their combination with genetical material or its precursors, if (as seems likely) this is essential to those at least which function through biological alkylation. Twenty years ago I suggested (Haddow, 1938) that the primary step in carcinogenesis might be an inhibition of the normal growth mechanism, leading to the adaptive formation of a new cell variant, released from the inhibitory influence of the agent which provoked it. The newer interpretation is that the primary step may be the inhibition of certain fundamental processes of genetical or enzyme synthesis, followed by the generation of a new self-duplicating fibre or template, chemically modified and hence genetically also. The permanent reorientation of genetical characters, under the impress of chemical modification, recalls the manner in which the secretion of a normal globulin may be replaced by that of an immune body, through the influence of a foreign antigen—as in the views of Burnet & Fenner (1949), that antibody formation is the consequence of an inherited change in the pattern of synthesis of serum globulin, a change enduring through repeated fissions long after the disappearance of the agent which brought it about.

A vital question is the chemical nature of the hypothetical receptor or receptors. From cytological and other evidence—as for example that the initial combination of many alkylating agents with genetical material probably occurs in the interphase between mitoses (Revell, 1952)—it is tempting to suspect that the action may interfere with some stage in deoxyribonucleic acid synthesis. Also, many such agents appear to operate predominantly upon the so-called heterochromatic regions in the chromosome, which are regions having a high nucleic acid charge in the resting nucleus. Bearing in mind these and many other considerations, and above all Mather's interpretation of the genetical activity of heterochromatin as the seat of quantitative inheritance (Mather, 1944, 1949), the impression remains, in spite of much difficulty, that heterochromatin may include a unique target so far as concerns the chemical induction of variations in the timing and extent of growth and differentiation. It is not perhaps a coincidence that Koller many years ago, from a cytological analysis of human tumours, suggested that the increased rate of division in their cells might be attributable to a quantitative change in nucleic acid synthesis resulting from alteration in the heterochromatic regions (Koller, 1943).

Almost as old as the experimental study of cancer is the question whether the process of carcinogenesis should not be regarded as a special case of biological mutation,—whether every mutagen is a carcinogen, and every carcinogen a mutagen, as was raised by Strong (1949). Whatever the relationship of the carcinogenic and mutagenic mechanisms, there is no doubt that each can shed light on the other. From a cytogenetical analysis of the action of carcinogens in *Drosophila melanogaster*, with special reference to 1:2-3:4-diepoxybutane, Bird & Fahmy (1953) first suggested modification in gene pattern of such a kind as to prevent normal reproduction, the disabled gene reproducing itself either partially or erroneously, or not at all. The general biological evidence has for some considerable time pointed to the possibility that the cancer cell represents a somatic mutation by loss, and recent work would appear to support this—although by no means as yet conclusively—and to suggest that the deficiency is genetical or enzymic in nature. Certainly it is entirely feasible that the loss of enzyme systems normally concerned in controlling the synthesis of substances essential to cell division could result in the unregulated synthesis or accumulation of such substances, and so convert the normal cell from a state of nutritional dependence to one of self-sufficiency and unimpeded growth. The principle proposed is by no means novel. Reference has already been made (see section 2) to the suggestion of the Millers that combination of an azo carcinogen and an autosynthetic protein could result in the gradual removal of this protein from the cell and its descendants, an autonomous tumour thus arising from a permanent alteration or loss of proteins essential for the control of growth. Potter (1950) also believed there are certain enzymes of strategic importance in limiting the growth of normal cells, the loss of which may mark the conversion from normal to malignant. In a specific case, Carruthers & Suntzeff (1952) found that a polarographically reducible substance, characteristic of epidermis, is lost in the malignant transformation of this tissue by methylcholanthrene. The general concept is not dissimilar to that put forward by Osgood (1957) for the aetiology of the leukaemias and lymphomata. In addition to his work on hepatocarcinogenesis (see section 2), Weiler (1956b, 1956c) has also studied the antigenic differences between normal hamster kidney and stilboestrol-induced kidney carcinoma, and the deficiency hypothesis has many points in common with the immunological concept put forward by Green (1954, 1957; see also Green, p. 101 of this number of the Bulletin), of the absence of immunological identity in neoplastic cells. In spite of these interesting and largely consistent trends, the present state of knowledge by no means permits any final decision. To take an example, Feldman & Sachs (1957) believe that the capacity of mouse tumours to grow in unrelated mice is not accompanied by a decrease in antigenicity, but is due to their increased resistance to the immune response, antigenicity being in fact increased. Hence the apparent loss of antigens on tumour transplantation may be spurious, and other possibilities must certainly be considered, such as a broadening of " acceptance ", or a return to some more general kind of " embryonic " protein. Before leaving the matter of deficiency in tumour tissues, and in view of the early and classical recognition of the general similarity of cancer cells and embryonic cells, it may be of value to recall very much older opinions on the absence or slow appearance during embryological development of the enzyme systems concerned in the transformation of the purines, as a feature characteristic of active growth and synthesis (Needham, 1931). These raise the problem as to which normal cell type should be regarded as the true homologue of the cancer cell—whether the differentiated adult cell, the differentiated embryonic cell, or the undifferentiated pluripotent embryonic cell. Whichever viewpoint is most justified, there is no doubt of the need for a more systematic study of enzyme distribution in embryonic and

malignant cells than has been available hitherto. It will not escape attention that the possible role of enzyme-protein deletion in carcinogenesis would indicate a specific difference between normal and malignant cells, with implications as to the means by which the process might ultimately be controlled —whether by substitution of the enzyme itself or of the reaction products of the substrate. That such a possibility is not entirely theoretical—even though it may be far from realization—is shown by the increasing number of model examples of the principle, in both biology and medicine. Meantime, it can fairly be claimed that the past ten years have seen at least an approach towards certain correlations, as for example between the structure and properties of given carcinogens, their combination at certain chromosome regions, the cytological repercussions, and the cytogenetical consequences which thus ensue.

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THE BIOLOGICAL EXAMINATION OF CARCINOGENIC SUBSTANCES

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1 Species of animals suitable for investigations
2 The influence of sex
3 Route of administration
4 Media or solvents
5 Initiating and promoting activity
6 Purity of compounds
7 Doses employed
8 Duration of experiments
9 Positive and negative controls
10 Indicative tests
11 Interpretation of the biological findings
12 Limitations in the application of the result
13 The groups of animals necessary for detection of carcinogenicity
References

"Prevention is better than cure" is a truism which is applicable with more force in the field of cancer than in most other diseases. As cancer is sometimes induced by certain chemical compounds, it can be prevented by avoiding contact with these substances. In view of this, the determination of carcinogenic hazards in the environment is of obvious importance in reducing the cancer incidence. This review is based on a report (Boyland, 1957) which was presented as a basis for discussion. The quantitative aspect of the problem is not emphasized, because small amounts of proved carcinogens are dangerous and it is only prudent to assume that there is no safe dose for carcinogens.

I have previously recommended that any substance which produces cancer in any species of animals at any dosage level should not be allowed in human food. This recommendation was made (at Bad Godesberg in 1954) not only on the grounds of prudence but also as a practical measure. If there is a safe dose of a carcinogen this could be determined only by using very large numbers of animals. The discussion in this review shows that it is difficult and expensive to determine whether any substance is carcinogenic; to decide whether there is a dose which does not cause cancer in, say, 1% of the population is not feasible. The principle that there is no safe dose of any carcinogen has been generally accepted in Europe, as the basis for regulations governing the use of food additives.

Barnes & Denz (1954) have assumed that chronic toxicity tests over three months are adequate and suggest that longer tests are "studies of rodent geriatrics". Carcinogenicity can be seen, however, only after prolonged tests. We do not know how much of cancer in man is due to environmental factors and how much is spontaneous. Even if only a few cases of cancer were produced by the use of food additives known to cause cancer in animals, surely everything should be done to avoid these. The routine investigation of new substances —which have been introduced or proposed as food additives or for other purposes—for carcinogenic activity will increase knowledge of carcinogenesis. Greater understanding of the processes of cancer induction might lead to prevention of cancer which is now considered spontaneous, but which may be caused by endogenous carcinogens.

Much actual experimentation and thought is needed to improve and develop methods for testing for carcinogenic activity, and they cannot be rigidly specified at the present time.

1. Species of Animals Suitable for Investigations

For most practical purposes we require to know whether a substance will produce cancer in man. There is evidence indicating that 4-aminodiphenyl, asbestos, benzidine, 2-naphthylamine, certain mineral oils, chimney-soot, cigarette smoke, coal-tar, radioactive materials and sodium arsenite cause cancer in man. The carcinogenic action of all these materials, except asbestos and arsenite, has been demonstrated in at least one animal species. Sodium arsenite is particularly interesting, as although it induces cancer in man, it has not produced cancer in any experimental animals in which it has been tested. A less extreme example of "species" difference is found with 2-naphthylamine, which causes bladder cancer in man and in dogs (Hueper & Wolfe, 1937) but not readily in other species of experimental animals.

The choice of experimental animals is limited to vertebrates, because cancer, as distinct from tumours, is rarely if ever seen in invertebrates. Although fish, amphibia and reptiles develop malignant tumours, they have been used only occasionally in the study of carcinogenesis. Tumours have been induced in several species of birds (cf. Peacock, 1958), and chickens might be used if a non-rodent species were required. The animals used most frequently in experimental carcinogenesis are mice and rats. Mice, hamsters and rats are small, cheaply housed and fed, and develop cancer more quickly than other species. The latent period of carcinogenesis in a particular species is probably proportional to the life-span of the species; the carcinogenic process would seem to be some thirty times more rapid in mice than in men.

Many pure lines of mice are available and might be more suitable for chronic toxicity tests than are stock mice. Mice of inbred strains have the advantage that the incidence of spontaneous tumours in the strain is known and reasonably constant. A possible drawback to the use of inbred animals for testing new substances for carcinogenic action is that the strain chosen might be specifically resistant to the carcinogenic action of a particular compound. There is often no correlation between the incidence of induced and spontaneous tumours—for example mice of the A strain have few spontaneous hepatomata but develop them readily on treatment with *o*-aminoazotoluene. By using stock mice in which there is some genetic variation, the chance of all the members' of the group being resistant is reduced. For quantitative comparison of activity of related carcinogens, pure-line animals usually give more consistent results (cf. Kreyberg, 1934; Boyland & Warren, 1937; Shimkin & Andervont, 1940).

First generation (F_1) hybrids of pure-line animals have many advantages, including vigour, the sensitivity of the parent strains and constant genetic make-up. The animals should be of strains which do not contain the milk factor or for any other reason develop spontaneous tumours at an early age, because such tumours complicate the assessment of the activity of an applied carcinogen.

Because of the difference of different species in susceptibility to carcinogens, substances should be tested on two species of animals. Some American workers consider that one of these species should be a non-rodent and that the non-rodent of choice is the dog. The cost of adequate tests on dogs both in material and in time is prohibitive. In view of the difference in reaction between mice and rats to carcinogens, tests on these two species are generally recommended at the present time.

2. The Influence of Sex

The incidence of induced tumours is frequently different in the sexes. Thus hepatomata are more readily induced in female mice of strains A, C3H, C and C57 black strains than in male animals of the same strains (Andervont, Grady & Edwards, 1942), and male mice of strain A are more susceptible to the action of small doses of 3 : 4-benzopyrene than are female mice (Leiter & Shear, 1943). Similar differences occur in human beings; the incidence of cancer of the bladder or of the rectum is about three times as great in men as in women. Because of this sex difference in response to carcinogenic stimuli, new agents should be tested on animals of each sex.

3. Route of Administration

In addition to skin-painting—the method of application in the first successful experiments in carcinogenesis—materials may be administered by inhalation, oral administration (either in food or by instillation into the stomach), injection by intradermal, intramuscular, intraperitoneal, intravaginal or intravenous routes, or implantation into tissues or organs.

Skin-painting is particularly suitable for testing products such as oil, coal-tar derivatives and substances used in cosmetics, ointments and creams, which might be applied to human skin. Skin tumours can be observed as they appear; this facilitates the experiments.

Inhalation and possibly nasal insufflation are methods of application of materials which have not been widely used in the past, but are of obvious importance in view of the great increase in the incidence of lung cancer in man. Aerosols of carcinogens are difficult and dangerous to handle, but bronchial carcinomata have been produced in rats with aerosols of beryllium (Vorwald, 1956) and radioactive strontium (Lisco & Finkel, 1954), but not with aerosols of carcinogenic hydrocarbons (Kuschner, Laskin, Cristofano & Nelson, 1957; Boyland, Druett & Pratt, unpublished observations).

It could be argued that the appropriate method of testing compounds to be used in food, or drugs to be taken orally, would be by oral administration. Such substances should indeed be tested in this way, but testing by parenteral injection is more convenient and stringent. Some carcinogens (e.g., dimethylaminoazobenzene) may be decomposed on being kept in mixed food. The technique of injecting material into the stomach of animals with a syringe and blunt needle or plastic tube ("forced feeding") is an effective way of administering substances. Tests of a number of carcinogenic water-soluble dyes, such as Light Green SF (Schiller, 1937), which proved to be carcinogenic on injection into rats, have formed the basis on which such dyes have been prohibited as food additives in Europe. Although the dyes may not be absorbed from the alimentary tract of the experimental animal, they might be absorbed from the human alimentary tract, or absorption might be facilitated by other substances present in food or by the presence of some lesion in the alimentary tract. Food additives should be tested both by injection and by feeding, but the injection test is the more stringent and desirable.

Whereas cancers of the stomach and other parts of the alimentary tract occur frequently in man, they do not do so in untreated rats or mice. The human alimentary tract may therefore be more sensitive than the animal gut to carcinogenic stimuli. If substances used in food are to be tested by feeding, then an animal capable of developing cancer of the alimentary tract should be used. Subcutaneous injection of 3:2′-dimethyl-4-aminodiphenyl into rats induced intestinal tumours (Walpole, Williams & Roberts, 1952); this shows that the rat intestine is susceptible to carcinogenic stimuli.

Parenteral injection is the most widely used method of administration of carcinogens. Injections can be given frequently and are usually made twice weekly for a period of between 12 weeks and one year. Intravenous injection has been used extensively only with mice and rabbits. Intravenous administration of solutions or suspensions of carcinogenic hydrocarbons into mice usually induces cancer of the lung, in which organ the carcinogen is localized (see, for example, Shimkin & Lorenz, 1942).

The implantation of pellets containing suspected carcinogens into the bladder of rodents has been of value in the investigation of bladder cancer (Maisin & Picard, 1924; Bonser, Clayson, Jull & Pyrah, 1952; Allen, Boyland, Dukes, Horning & Watson, 1957). With this method the possibilities of metabolism of the administered compound are reduced, so that some evidence as to whether a substance is a carcinogen per se or only after metabolic change can be obtained. Thus, Bonser *et al.* (1952) showed that 2-naphthylamine did not induce bladder cancer under such conditions, but that one of its metabolites, 2-amino-1-naphthol, did do so. Experimental bladder implantation has not been widely used, but it is useful in studying mechanisms of carcinogenesis. This method is being used in my laboratory to investigate potential carcinogens present in cigarette smoke. The carcinogen in cigarette smoke appears to act directly on the bronchial epithelium where the possibilities of metabolic change are small—in this respect the bladder epithelium may resemble the bronchial epithelium.

The route of administration of a substance to be tested should depend upon the proposed use of the product. Thus, for example, food additives should be tested by administration in food or into the stomachs of animals, contraceptives by intravaginal application, cosmetic ingredients by skin-painting. In addition to this, however, the material should be tested by injection, in order to overcome differences in absorption. If only one route of administration is used, this should be parenteral.

4. Media or Solvents

Substances soluble in water are probably best administered in aqueous solution; even insoluble substances, such as the polycyclic hydrocarbons, have induced tumours on injection as aqueous colloidal suspensions (Boyland & Burrows, 1935). Aqueous solutions prepared for injection should be approximately isotonic with body-fluids, as injection of concentrated solutions of sodium chloride (cf. Tokoro, 1940), glucose (cf. Nishiyama, 1935) or fructose (cf. Takizawa, 1940) has induced occasional sarcomata, probably caused by the effect of the osmotic pressure of the injected solution rather than by specific chemical action.

Oil-soluble substances, such as the hydrocarbons, have usually been injected in oil solutions, but some batches of oils (e.g. arachis oil, cf. Walpole, Roberts, Rose, Hendry & Homer, 1954) are carcinogenic, so that solvent control experiments must be performed. Synthetic tricaprylin has advantages over the natural oils; it is a substance of known structure and, being saturated, is less chemically reactive than the natural oils. The results of Shimkin & Andervont (1940) indicate that it is the solvent of choice for carcinogenic hydrocarbons.

5. Initiating and Promoting Activity

Tests for cocarcinogenic action (cf. Salaman, 1958) should perhaps be performed in view of the observations of Setälä, Holsti & Lundbom (1957) that some emulsifying agents such as Tween 60 and other polyethylene sorbitan esters have cocarcinogenic or promoting activity. Bielschowsky & Lindsay (1956) showed that if mice are treated with urethane or small doses of methylcholanthrene which are initiators of carcinogenesis, followed by Tween 60, they develop skin cancer. This observation has been confirmed independently by Van Genderen, Van Esch & Vink (personal communication). Relatively few compounds have been tested specifically for initiating action and promoting action separately. If one agrees with Shubik & Sicé (1956) that "On the basis of present knowledge it is justifiable to condemn any substance that may possess promoting activity", then tests for initiating and promoting activity should be performed on substances which are intended for human use.

Tests of this kind are usually performed by skin application to mice. To determine promoting activity, mice should be given a single dose of a known initiator (urethane is probably the most satisfactory) and then repeated applications of the substance under test. Control series (i) with applications of a known promotor (croton oil) and (ii) with the substance without previous application of the initiator should be included (the last control would indicate whether the compound is a complete skin carcinogen).

Initiating activity is detected by treating mice first with the substance under test, followed by repeated application of a known promotor (e.g., croton oil). Control series should be included in which (i) no initiator is applied, (ii) a known initiator (e.g., urethane) is given in place of the substance under test.

6. Purity of Compounds

The purity of food additives should be controlled; many preparations used as food additives or for other purposes are either impure or are frank mixtures. In such cases each of the constituents should be tested separately and the composition of the mixtures controlled, so that they do not contain carcinogens as contaminants. New chromatographic methods have shown that some food dyes are mixtures (Reith, 1957).

7. Doses Employed

Sometimes, as with 5 : 9 : 10-trimethyl-1 : 2-benzanthracene (Badger, Cook, Hewett, Kennaway, Kennaway, Martin & Robinson, 1940), there is an optimum dose for carcinogenesis; this indicates that tests at different levels are desirable. One of the dose-levels employed should be the maximum which will allow long survival and reveal effects of chronic toxicity. The lower dosage level should be related to the concentration in which the substance will be used by man. The dose-level should be such as to give a wide margin of safety, and the conventional margins used are one hundredfold or one thousandfold (cf. Frazer, 1953). The use of these safety factors is rather vague and needs definition, and the assumptions need clarifying, particularly in deciding whether the ratio shall apply to an individual food constituent or to the total daily food intake. Recommendations have been made that tests at the lower dosage level should be carried out in order to detect general toxic effects, and these can be used to test for carcinogenic action at the same time.

8. Duration of Experiments

When weak or slowly acting carcinogens are being tested, the animals should be treated for some considerable period; this should be at least 12 weeks and can be one year or the duration of life of the animals. Even if treatment is stopped after one year, the control and treated animals should either be allowed to live out their life-span after treatment is finished or killed at some arbitrarily chosen time such as two years for mice or two-and-a-half years for rats. I think that termination of the experiment in this way is preferable to allowing the animals to live their natural life-span, but this is not universally agreed. If the test animals are sacrificed after some predetermined period, then other groups of animals should be observed for their life-span in order to determine if the treatment affects the duration of life. Recommendations have been made that treatment should be carried out over three generations of animals. Although this might reveal effects on fertility, it could not detect mutagenic action unless very large numbers of animals were used.

9. Positive and Negative Controls

The necessity of carrying out control experiments on the solvents used in carcinogenesis has been mentioned. In addition to such "negative" control tests, "positive" control series with a known carcinogenic agent, which is similar to the substance under test in chemical or biological properties, should be carried out. By this means the possibility of a false negative result, due to resistance of the treated animals, is reduced. Potent carcinogens, such as methylcholanthrene, should not be used as control substances, except in marginal doses, such as might be expected to induce cancer in half of the treated animals. With new classes of substances to be tested, it is often difficult to decide upon an appropriate substance for the positive control experiment.

For tests to show that a substance is carcinogenic, the incidence in the treated group of animals must be statistically significantly higher than in the control series. This can frequently be decided with greater economy of means if large groups of control animals are used and several substances are tested during the same experiment.

10. Indicative Tests

Up to the present no short-term tests have been devised which give a valid indication as to whether a substance is carcinogenic or not. As carcinogenesis is a radiomimetic

effect (cf. Boyland, 1952), substances which produce other radiomimetic effects are likely to be carcinogenic. The most widely studied effect from this point of view is the inhibition of growth by carcinogens first observed by Haddow (1935). The use of this test led to the discovery of new carcinogenic agents, such as the aminostilbenes (Haddow, Harris, Kon & Roe, 1948). The greying of hair of coloured mice after intradermal injection is another effect which parallels carcinogenesis to some extent (Boyland & Sargent, 1951). Carcinogens are often mutagenic (Boyland, 1954) and cause chromosome damage (Koller, 1954), so that any substance with these properties should be regarded as possibly being carcinogenic. None of these tests, however, can give more than an indication that a substance may be carcinogenic. The occurrence of such effects in plant materials is less significant than when they are seen in animal tissues. Thus maleic hydrazide produces chromosome damage in plant cells, but not in animal cells, and is probably not carcinogenic. On the other hand urethane produces chromosome damage in animal cells, but not readily in plants, and induces tumours in mice.

11. Interpretation of the Biological Findings

Many substances are weak carcinogens. They may be designated as weak because: (i) they produce cancer in small proportions of animals, even with optimal doses, (ii) they are slow in acting, or (iii) large doses are required. These aspects —the proportion of animals affected, the time taken to produce the effect and the amount of material required—should be considered separately. If the substance produces tumours in only a small proportion of treated animals, then relatively large groups of animals must be used in both the control and treated groups in order to obtain a significant result. If the carcinogen is slow in action, then groups of animals must be large enough for sufficient to survive until the time of tumour incidence to give a significant result. The numbers of animals necessary to obtain statistically significant values for the incidence of cancers—assuming that no tumours occur in the negative control groups included in Table I—refer to the animals surviving to "tumour age", a term which needs defining. If an incidence of cancer in 20% of the animals is to be detected, when no tumours appear in the controls, then each group should contain at least 20 animals. In this range the number of animals required would have to be doubled in order to produce a twofold increase in sensitivity of the test. Tests would probably be effective if 20 animals were in each group, provided that most of them survive to the "tumour age".

The expression of significance ($P = 0.025$) used means that the result should appear only once in 40 times if the effect were due to chance. If 40 inert substances are tested, one might expect one of them to give a positive result by the random distribution of the effects produced by the control treatment. In practice the control treatment often induces a certain number of tumours, so that the possibility that a false positive would appear by what might be considered to be a "freak" distribution cannot be neglected, particularly when a large number of substances are tested.

Some substances, which are perhaps loosely described as carcinogenic, produce lesions such as adenomata that are not malignant; but in making recommendations about substances of this type it must not be forgotten that adenomata or papillomata may be precancerous.

TABLE I. DETECTION OF CARCINOGENICITY IN EXPERIMENTS WHERE EQUAL NUMBERS OF EXPERIMENTAL AND CONTROL ANIMALS REACH TUMOUR-BEARING AGE AND WHERE NO TUMOURS APPEAR IN CONTROLS

Minimum tumour incidence which can be regarded as significant, i.e. $P_t = 0.025$

Number of animals in each group	A. Calculated from Fisher's exact method of 2 × 2 tables*		B. Calculated from limits of expectation of binomial and Poisson distribution†	
	Animals with tumours		Animals with tumours	
	Number	Percentage	Number	Percentage
3	($P = 0.025$ not attainable)		3	100
4	4	100	3	75
5	4	80	3	60
10	5	50	4	40
15	5	33	4	27
20	5	25	4	20
25	5	20	4	16
30	6	20	4	13
50	6	12	4	8
75	6	8	4	5
100	6	6	4	4

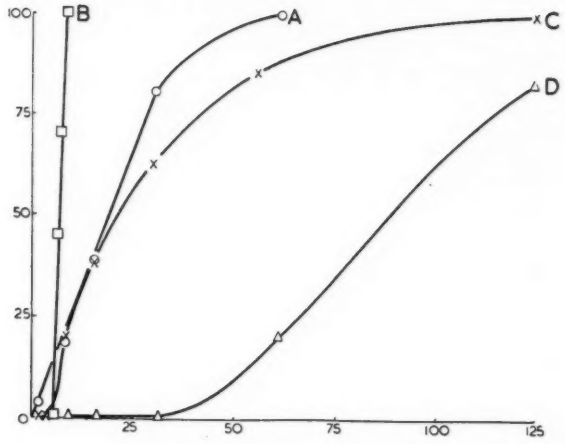
* Table VIII, Fisher & Yates, 1953.
† Based on Table VIII, 1, Fisher & Yates, 1953.

I have previously suggested (Boyland, 1953) that some carcinogens differ from other toxic agents in that no threshold or safe level exists, and Druckrey (1951) has emphasized that carcinogens have cumulative and irreversible effects. Although these generalizations cannot be proved for man, they are the only reasonable assumptions on which recommendations can be made. Study of the quantitative pharmacology of carcinogenesis (Boyland & Warren, 1937; Bryan & Shimkin, 1940, 1942; Lettinga, 1937) has shown that the induction of tumours with 1:2-5:6-dibenzanthracene expressed in probits varies linearly with the logarithm of the dose. If the dose-response relationship for 1:2-5:6-dibenzanthracene is plotted linearly (fig. 1), it appears as a straight line passing through the origin, whereas methylcholanthrene and 3:4-benzopyrene have S-shaped curves. Extrapolation of the data indicates that doses of 0.5 μg. of 1:2-5:6-dibenzanthracene would induce cancer in one mouse in each 500 treated. If there is no safe dose for this carcinogen, which is one of the few which have been investigated quantitatively, then it is only prudent to assume that there is no safe dose for carcinogens which have not been investigated quantitatively.

The amounts of different carcinogens necessary to induce cancer in 50% of the treated animals (ED_{50}) vary greatly. Thus some ED_{50} values are as follows: 1:2-5:6-dibenzanthracene, 20 μg.; 20-methylcholanthrene, 20 μg.; 3:4-benzopyrene, 80 μg.; 4-dimethylaminostilbene, 100 mg.; 4-dimethylaminoazobenzene, 500 mg.

It has been suggested (Boyland, 1953) that the usual relationship of the effective doses of drugs being proportional to the body-weight or surface area of the animal does not necessarily hold with carcinogens, because cancer can develop from a few cells or possibly from a single cell. In the first place the carcinogen may be localized, in which case it would affect

FIG. 1. DOSE-RESPONSE RELATIONSHIPS FOR THREE CARCINOGENS, AND MORTALITY DUE TO POTASSIUM CYANIDE



A: 1:2-5:6-dibenzanthracene
B: potassium cyanide
C: 20-methylcholanthrene
D: 3:4-benzopyrene

Abscissae for A, C and D: dose (linear scale) (μg. carcinogen/mouse); for B: mg. potassium cyanide/kg. body-weight
Ordinates for A, C and D: number of mice with cancer (%) (linear scale); for B: mortality (%)

the same number of cells were it localized in a large or a small animal. If cancer arises either from a single cell or from a relatively small number of cells, the probability of cancer's being produced should be independent of the size of the animal, even if the carcinogen is not localized. The chance of cancer's developing is presumably dependent on the number of "effective encounters" between carcinogen molecules and susceptible cells, and there is no reason why the number of these should be dependent on the number of cells exposed. If there are approximately 2,000 times as many cells in a man as in a mouse, then each individual human cell would have only one chance in 2,000 of being affected by the same dose of a carcinogen as a cell of a mouse given the same dose. As there are 2,000 times as many cells in a man as in a mouse, the final probability of one cell in the whole animal being affected will be the same. This would mean that the same dose of carcinogen would be as effective in a man as in a mouse, ignoring species sensitivity of individual cells. This is similar to infection, in which a single cell of a pathogen or a small amount of virus might lead to the death of a large or small animal. In infection the invading pathogen multiplies in the host; in cancer the original cancer cell or group of cells proliferates, but the original cancer is independent of the size of the host.

12. Limitations in the Application of the Result

Although there is no indication that safe doses of carcinogens exist, yet some substances known to induce cancer in animals cannot be considered dangerous. Thus relatively large pellets of paraffin wax induce papillomata when implanted in the bladders of rats. Similarly, plastic materials implanted into the subcutaneous tissues of rats induce sarcomata (Oppenheimer, Oppenheimer, Danishefsky, Stout & Eirich, 1955). These results cannot, however, be considered as indications that plastic and papers treated with paraffin wax would be dangerous to man when used as packaging materials for food.

The induction of occasional tumours by injection of concentrated solutions of sodium chloride or sugars has already been mentioned, but these experiments cannot be taken as contra-indications for the use of these substances by man. The results of tests of carcinogenic action in animals must be considered in relation to the use which the material will have before it is said to be dangerous.

13. The Groups of Animals Necessary for Detection of Carcinogenicity

The recommendations made in this discussion indicate that for effective detection of carcinogenicity it is advisable to take groups of 20 animals and to provide two species, two dose-levels, two routes of administration and both sexes. This means that 320 animals in the treated series, and 160 in each series of negative and positive controls, should be kept under observation. If initiating and promoting action are to be determined, then more animals would be needed. Such numbers are possibly idealistic and impracticable, but the investigator must decide if and how the numbers can be reduced. If the numbers are to be reduced, I would suggest that the lower dose-level might be omitted. If numbers must be further reduced, then the route of administration other than injection might be abandoned. If a substance is a potent carcinogen, then its effect can be seen by using a few animals of any sex or strain. The problem with food additives and ingredients of cosmetics, contraceptives and other products used in present-day life is how to decide that a substance is harmless, meaning that it does not induce cancer even in a small proportion of treated animals.

If it is accepted that substances which are known to be carcinogenic by injection should not be used in food, drink, cosmetics, contraceptives, detergents or other products freely available to the general public, then it should be sufficient to test them by injection only. Some substances have multiple uses, for example, the same dyes are used in food and cosmetics, and some emulsifying agents are used in food, cosmetics and contraceptives. No substance known to induce cancer should be freely available to the public. On the other hand, valuable medicaments which are known to be carcinogenic should be available for use only under the direction of a physician, who should be in a position to decide whether the advantages justify the risk of using such dangerous remedies.

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THE MECHANISM OF CHEMICAL CARCINOGENESIS

With Particular Reference to the Time of Development of Irreversible Changes in the Epithelial Cells

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1 The latent period in cancer induction
2 Early changes in carcinogenesis
3 Results of skin grafting experiments
4 The multicellular origin of cancer
References

An established cancer is a mass of abnormal cells, and it is therefore not surprising that, since the experimental induction of cancer became possible, much effort has been expended in attempts to discover the nature of the change in the affected cells themselves, on the postulate that a chemical carcinogen may enter, or become attached to, these cells and produce decisive intrinsic changes in them. The greatest problem in such investigations is the lack of any criterion by which a cell in a phase intermediate between normal and neoplastic can be recognized.

1. The Latent Period in Cancer Induction

All chemical (and radiation) carcinogens take a considerable time to declare their effects. After a certain dosage, which may be as little as a single application of the potent carcinogen 9:10-dimethyl-1:2-benzanthracene (DMB), cancer may develop a long time afterwards, even though the epithelial cells may be histologically, histochemically and cytologically normal for a period in the meantime. It is reasonable to regard the total disease process of cancer, in contradistinction to the developed tumour, as starting from the time that effective carcinogenic treatment has been completed. Already during the so-called latent or induction period, there has been set in motion a train of events which will lead to the appearance of a declared tumour, and it is of the utmost importance in the study of aetiology to try to find out what is happening during this latent period.

There are two main hypotheses to explain the latent period: (i) that the carcinogen produces in the first place latent tumour cells which cannot be recognized as such by any methods at present available (the "initiating" process of Friedewald & Rous, 1944), and (ii) that the primary effect of the carcinogen is a "field" change affecting all the elements in the treated area, including the supporting and nutrient stroma, the neoplastic quality arising later in the epithelial cells as a result of the altered metabolic conditions to which they have become exposed. The first hypothesis has been more widely accepted, and the evidence in its favour is closely linked with problems of cocarcinogenesis, discussed by Dr Salaman in this symposium (Salaman, 1958). It is the purpose of this article to summarize some of the evidence which seems to me to make the second view more tenable. It will not be possible in the available space to survey adequately the relevant literature, and apologies are offered for the disproportionate attention which will be paid to the work of the author and his colleagues.

2. Early Changes in Carcinogenesis

When mouse skin is treated with a carcinogen such as 20-methylcholanthrene (MC), epithelial hyperplasia with stratification and keratinization is found within a few days. This might be the result either of growth-stimulation, or of regenerative over-repair following damage. That the latter may be the explanation is suggested by the fact that suitable concentrations of DMB result in total destruction and necrosis of the epidermis, followed by regenerative spread from the periphery of similar stratified squamous epithelium. In passing, it should be noted that epidermal regeneration is a very rapid process, as Gillman, Penn, Bronks & Roux (1955a) have shown in their recent studies on wound healing. It is often forgotten that many normal tissues, including epidermis, can grow much more rapidly than tumours when necessary, e.g., in sites from which skin grafts have been taken or in superficial scrapes and abrasions. When wound healing is complete, growth ceases. What stops it? It is difficult to see from where such control can come except from the local stroma; if it were a generalized systemic agent it would tend to stop the healing of wounds in other parts of the body.

The immediate epidermal hyperplasia evoked by carcinogens shows little additional change—it may even regress—until the appearance of tumours themselves. In the dermis and subcutis, on the other hand, there is throughout the latent period a progressive alteration of the collagen and elastic fibres, and the rate at which this change advances is correlated with the potency of the carcinogen used (Orr, 1938).

3. Results of Skin Grafting Experiments

A series of grafting experiments gave support to the view that the effects of the carcinogen on the supporting tissues were all-important (Billingham, Orr & Woodhouse, 1951; Marchant & Orr, 1953). When mice have received 12 weekly applications of MC in acetone and are observed without treatment thereafter, almost all of them may be expected to develop local skin tumours a few weeks later. This would imply, if the action of MC were intrinsic on the epithelial cell, that the epidermis is at this stage well advanced towards neoplasia. Nevertheless, when grafts of pure epidermis so treated were transferred to a bed on the corresponding site of the untreated side of the same animal, no tumours developed. The same finding held for thin Thiersch grafts, including the epidermis and superficial epidermis. It was only with thick Thiersch or whole skin grafts that the capacity to produce tumours could be transplanted. Even when such epidermal grafts were subsequently given a full cocarcinogenic course of croton oil, papillomata appeared only in the small number also obtained on normal skin which has received no "initiating" carcinogenic treatment.

On the other hand, if the site which has received the 12

weekly applications of MC is deprived of its epidermis, and is either allowed to resurface itself by natural processes or grafted with epidermis from another part of the skin which has not received carcinogenic treatment, tumours appear in about the same incidence as might be expected in intact skin. It might be argued that the cells forming such tumours are derived by regenerative proliferation from the roots of the hair follicles, which are left in situ when Thiersch grafts are removed, but if so it is surprising that the incidence of trichoepitheliomatous or sebaceous differentiation in these tumours remains low. Some experiments with DMB give almost decisive evidence on this point (Orr, 1955).

When mouse skin is treated with 0.5% DMB in acetone, the epidermis and hair follicles are totally destroyed in a few days. Sometimes the superficial dermis is also damaged and subsequently sloughed, but the damage is often confined to the epithelial structures. Thereafter, regenerative epidermis grows in from the periphery, and new hair follicles and sebaceous glands are formed by differentiation *de novo* from the regenerated epidermis. That new hair follicles can be so formed in healed defects in rabbit skin has been shown by Breedis (1954) in experiments unconnected with carcinogenesis as such. Gillman, Penn, Bronks & Roux (1955b) believe that the hair follicles are completely destroyed and reformed during MC treatment, a view with which I am inclined to agree, but the evidence is not so decisive as with DMB.

The skin so treated with DMB will later go on to the production of tumours, especially if it receives subsequent cocarcinogenic treatment. The epithelium forming these tumours seems unlikely to have come into direct contact with DMB, particularly in the central parts of the treated area, in which most of the tumours arise. It is, of course, impossible to define with certainty the limits of the carcinogen-treated area, as there is possibly some spread of DMB beyond the ostensibly treated region. On the other hand, the demarcation between the epilated zone and the fully furred skin is generally sharp. If we assume, however, that the cells of the tumour are derived from peripheral cells which received a minimal dose of DMB, the interpretation of these results in the light of the grafting experiments and in terms of the latent-cell hypothesis becomes complicated. It would be implied that peripheral latent cells are capable of proliferating to form surface epidermis, then differentiating to form hair follicles, then dedifferentiating again to squamous epithelium, and remaining latent in spite of all this growth activity. It should also be noted that during these experiments no tumours have been observed in the peripheral region itself. A fundamental fact arising from all these observations is that the vast majority of the tumours arise well within the original treated area, the permanent component of which is the stroma.

While there is thus strong evidence that the epithelial-stromal relation is all-important in carcinogenesis, it is probably fallacious to relate it, as has sometimes been done, to a decreased resistance of the stroma to the downward spread of the epithelium. The hair follicle itself descends through a normal or a thickened sclerotic corium without obvious impediment, as has been pointed out by Wolbach (1951). Cortisone administered during carcinogenic treatment has been found both to increase (Piccagli, Hermann, Frank, Rothstein, Morrill & Sulzberger, 1954) and to depress (Gillman, Penn, Bronks & Roux, 1955c) the yield of tumours. It seems more likely that the neoplastic and ultimately invasive growth of the epidermal epithelium is to be attributed to factors affecting its nutrition, or possibly its enzymic or hormonal control, all of which must be, to some extent at least, dependent on the integrity of the underlying dermis. I have previously suggested that vascular inadequacy may be of importance, and *intra-vitam* staining with phenol red shows that a locally decreased pH may precede the appearance of tumours (Orr, 1937).

4. The Multicellular Origin of Cancer

The conception of latent cancer cells, with its implication that cancers arise by multiplication from isolated single cells, is at variance with the straightforward interpretation of histological findings. Willis (1953) has emphasized this in relation to the inception of various types of cancer, pointing out that the appearances are those of a generalized change in a field of epithelium. Most histopathologists who have examined relevant material would agree with him, but it is a point that continues to escape the attention of cancer research workers who regard all inferences based on histological observations with suspicion. The picture seen in carcinoma in situ could not be explained on the basis of multiplication from a single cell and, while not all examples of carcinoma in situ can be definitely shown to go on to invasion, many can. In relating cellular mutation to carcinogenesis, many authors think it necessary to postulate multiple changes; in this respect histology would appear to offer more support to the concept of Fisher & Hollomon (1951) of colonies of six or seven adjacent mutated cells than to the theory of successive changes in a single cell which has been advanced by Nordling (1953) and Armitage & Doll (1954), and is preferred by Burnet (1957).

Apart from the use of cocarcinogens, most carcinogens require repeated application to be effective. Even DMB produces tumours not only more abundantly, but also more rapidly, when this is done. It is difficult to understand why this should be so if the essential "initiating" process is due to an immediate intrinsic effect on isolated epithelial cells.

Brues (1955) gave good reasons for regarding the mechanism of radiation carcinogenesis as indirect and not determined by immediate cellular changes, but stated that this was the opposite of chemical carcinogenesis. In the discussion which followed Brues's paper, Kaplan gave reasons for regarding an indirect mechanism as worthy of consideration in all carcinogenesis.

This article has been almost exclusively concerned with epidermal carcinogenesis in the mouse, but similar considerations in the wide sense are applicable to the experimental induction of tumours in several other organs and species, as well as to a number of types of human cancer.

It should perhaps be pointed out that the type of carcinogenic mechanism discussed here is not at variance with the experimental facts of cocarcinogenesis as observed by Berenblum & Shubik (1947) and subsequent investigators, but is concerned only with their interpretation.

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IMMUNOLOGICAL BASIS OF CARCINOGENESIS

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References

The immunological theory of cancer first put forward by Green (1954) has been considerably expanded and to some extent modified since then (Green, 1957, 1958). Although the theory was based on an immunological concept of chemical carcinogenesis, it attempted to embrace all causes of cancer and was concerned primarily with the fundamental nature of cancer and not with any particular mode of development. If cancer is an altered immunological state of the cell, then of course carcinogenesis must involve some kind of immune mechanism.

1. Loss of Immunological Identity in the Cancer Cell

To consider all the evidence is impossible in the space available and the publications mentioned should be consulted for full references and details. Owing to the condensed way in which the original paper (Green, 1954) had to be written there is still some confusion about the basis of the argument. Observations on the inhibitory effects of certain compounds on transplantable tumours were thought to indicate the mode of the chemical induction of cancer. Transplantation immunity was recognized as the normal (iso-antigenic) immunity common to all tissues, healthy or neoplastic. The fact that chemical carcinogens and related substances could increase this immunity was the first pointer to their possible immunological role. As they had little effect on spontaneous tumours it seemed, as would be expected, that iso-antigenic immunity was not evoked by such tumours. On the contrary, since cancerous tissue is, in general, more easily transplantable than normal tissue, this might indicate some degree of iso-antigenic loss. Thus we were led to the concept of antigenic deficiency as a feature of the cancer cell. It was not postulated that iso-antigenic loss was a major feature but rather that this was a pointer to the loss of organ-specific antigens, a feature which we now regard as the essential factor in neoplasia. How these ideas developed will now be more fully considered.

2. Nature of Tumour-Inhibiting Action of Polycyclic Hydrocarbons

The key observation was that the tumour-inhibiting (TI) action of carcinogenic polycyclic hydrocarbons[1] was not the expression, as previously thought, of a depressant effect on somatic growth generally, but a specific effect on the transplanted tumour. This was revealed by finding compounds in pitch coking oil, which did not depress body growth but strongly inhibited transplanted tumour growth. These compounds, at any rate naphth-2′:1′-1:2-anthracene and 11:12-benzofluoranthene, which have been most widely used, are probably non-carcinogenic, and are less soluble than most polycyclic carcinogens, a feature suggesting that local retention at the site of injection might be a factor in their TI effect. The related more soluble carcinogens had no TI effect at similar dosage (around 2 mg.) but the most insoluble, 1:2-5:6-dibenzanthracene, was active and at this low dosage did not depress body growth. It was then found that the more soluble polycyclic carcinogens (e.g. 9:10-dimethyl-1:2-benzanthracene) also showed TI properties when injected in repeated small doses. It gradually emerged that specific inhibition of transplantable tumours was a property of polycyclic carcinogens and a few related non-carcinogens. To what was this property due?

[1] See Haddow, A. (1938) *J. Path. Bact.* **47**, 581.—ED.

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It was clear that it involved a general effect on resistance, as no trace of the compound was found in the tumour though there was a little in the draining lymph nodes and sometimes the spleen. Spontaneous tumours and highly strain-specific tumours were relatively unaffected. Neither was every transplanted tumour affected, as only half, on an average, of a group regressed completely, and a small proportion was completely unaffected. All this suggested that resistance to tumour growth was being augmented by an increased production of iso-antibody and that, where there was no incompatibility between tumour and host, such antibody, if formed, was ineffective. The complete abolition of the TI effect by simultaneous treatment with cortisone strongly supported this view, particularly as such treatment had a general toxic effect. Further evidence was that plasma-cell proliferation, a feature of any immune reaction to transplanted tumours, was induced by these TI compounds.

The question remained whether the increased resistance was due to a direct stimulation of the reticulo-endothelial (RE) system. This seemed an unlikely property, as carcinogens tend to depress antibody production to foreign proteins. It seemed more likely that the carcinogen, by binding with tissue protein, formed an auto-antigen, behaving immunologically like an iso-antigen. This seemed all the more likely since protein-binding was a known property of many carcinogens. Work on this subject, particularly by Miller & Miller (1952), Heidelberger & Weiss (1951), and Heidelberger & Moldenhauer (1956), has been discussed in detail (Green, 1958) because of its important bearing on our theory. In view of the wide variety of pure chemicals, from simple metals to complex hydrocarbons, which form antigenic complexes, particularly in the skin, it would be most surprising if some of the chemical carcinogens did not. They are indeed capable of haptenic modification, for artificial protein conjugates elicit specific antibody formation (Creech & Peck, 1952).

Another approach (Green, unpublished observations) is to attempt to alter the antigenic structure of tumour cells by exposure to carcinogens at the time of inoculation. In theory the transplantable homologous tumour, lacking iso-antigenicity in varying degrees, should be less susceptible to this procedure, and this seems to be so. On the other hand tumours growing only in inbred strains of mice are highly susceptible. When the inoculum is exposed to small amounts of TI compounds, growth restraint, varying from absolute, with a powerful carcinogen like 9:10-dimethyl-1:2-benzanthracene, to a prolonged delayed outgrowth with a carcinogen like dibenzanthracene or a non-carcinogen, such as 11:12-benzofluoranthene, occurs. The effect is specific and not obtained with hydrocarbons like anthracene, phenanthrene and picene. It may be immune in nature, for some growth may occur for around ten days before regression commences. The tumour is behaving like one injected into a mouse of another strain, i.e. as though having iso-antigenic properties. There are thus possibly two distinct ways by which carcinogens can affect iso-antigenic immunity: one by a remote action augmenting an immune reaction already operative, and the other by a direct action inducing an immune reaction not normally operative. Our interpretation is that a specific immunological change can be induced by certain chemical carcinogens.

a. Immune Reactions during Carcinogenesis

Nevertheless attempts to demonstrate specific antibody in carcinogen-treated animals have not met with success (see Green, 1958). Inconclusive evidence was found in the appearance in the serum of rabbits of panagglutinins for rabbit red cells after wide-spread painting with carcinogens. We also found evidence of a lipid-binding " antibody " in many such rabbits. The frequency of Kahn-positive sera was very high and there was a very significant correlation with the occurrence of panagglutinins. The Kahn reaction is of course non-specific in this connexion, but the evidence suggests that some lipid-precipitating substance with haemagglutinating properties appears in carcinogen-painted rabbits. Since tumour lipoprotein fractions strongly agglutinate rabbit cells (Green, 1957), it seems possible that an abnormal lipoprotein (? antibody) appears in the serum of rabbits during wide-spread skin carcinogenesis. If, as Burnet (1956) tentatively suggests, carcinogens elicit a sensitizing type of antibody bound by the affected tissues and not present in the serum, the difficulty of detecting them could be understood. That they will ultimately be detected seems likely.

Indirect evidence of immune changes is more plentiful. The fact that the spleen of carcinogen-treated animals contains a tumour-inhibiting factor is suggestive. Pollard & Russel (1953), whose results in mice we have discussed in detail (Green, 1954, 1958), demonstrated it in tissue culture, and Baruah (1956) showed that splenic pulp from carcinogen-treated rats prevents the outgrowth of a rat-tumour inoculum when mixed with it. A similar result was obtained with splenic pulp from rats with regressing tumours and there is ample evidence of tumour lipid-binding " antibodies " in such spleens (Green, 1957). It is noteworthy that in Pollard and Russel's work the splenic inhibition disappeared with the appearance of a tumour, suggesting that an exciting factor (? antigen) was present in the precancerous, but not in the cancerous, tissue. The nature of the antibody (if any) is not clear, but since the effect was limited to the tumour type induced by the carcinogen concerned, it may be a tissue-specific antibody rather than an iso-antibody.

b. Role of the Reticulo-Endothelial System in Carcinogenesis

Another aspect is the role of the RE system in chemical carcinogenesis. Green (1958) discusses this fully and points out that the failure to induce chemical carcinogenesis (as distinct from neoplastic change) in tissue culture suggests that a carcinogen needs to operate in organized tissues, and that to be effective some bodily resistance to its action is essential. What happens if the hypothetical immune response to a carcinogen-protein complex is diminished? Diminution can be achieved by RE blockade, by general irradiation, and by cortisone. Under appropriate conditions all have been shown to delay or prevent chemical carcinogenesis. There are several reports of RE blockade with trypan blue during azo dye feeding, the results of Iwase & Fujita (1955) being particularly interesting. The fewer liver tumours which arose were delayed and less malignant, but, nevertheless, the amount of protein-bound dye in the liver was not reduced. If such binding reflects the initiation of carcinogenesis, then RE blockade must have hindered the promoting stage, possibly by reducing the immune response. Similarly cortisone treatment does not affect initiation but may under certain conditions prevent the appearance of chemically induced skin papillomata (Ghadially & Green, 1954). This may not be an immunity response, however, for cortisone strongly depresses

epidermal mitosis (Green & Ghadially, 1951) and might in this way suppress the promoting stage.

There is evidence that superficial *x* irradiation in repeated small doses throughout carcinogenesis may suppress liver tumour development (Hoch-Ligeti, 1949). Here the RE stimulation which occurred may have augmented the immune response so that more precancerous cells were destroyed. In fact Micheel and his co-workers (Micheel, 1940, 1941a, 1941b, 1947, 1952; Micheel & Dörner, 1944; Micheel & Emde, 1942, 1944; Micheel, Emde & Dörner, 1942) delayed or prevented chemical carcinogenesis in mice by non-specific immunization with horse albumin and conjugates thereof. In theory enhanced immune reactivity could prevent carcinogenesis by iso-antibody destruction of all initiated cells, or accelerate it by a non-lethal antibody (? tissue-specific) attack causing a more rapid adaptation by loss of antigen. It is feasible that cholesterol accelerates carcinogenesis in the latter way, for though it does accentuate TI action, it also enhances the non-lethal immune response to lipoproteins and phospholipids.

General repeated exposure to *x* rays accelerates chemical carcinogenesis (Mayneord & Parsons, 1937). This may be due to an additive effect of two carcinogenic agents, or to a profound depression of RE activity which allows initiated cells to accumulate more rapidly. After recovery from *x*-ray damage, the immune response might be increased by the greater antigenic stimulus, and quicker adaptation by antigenic loss might result. Whatever the explanation of these *x*-ray, blockade and cortisone effects, they suggest that the RE system is intimately concerned in carcinogenesis and therefore that a body-defence mechanism is involved.

3. The Immunological Theory of Carcinogenesis

The immunological theory of carcinogenesis is that one or more of the cytoplasmic specific protein complexes which confer identity on the tissue are modified by carcinogen binding, the modified complex being self-replicating. Antibody is elicited to it and in due course adaptation, by loss of the modified identity protein, occurs. The new race of cells, now lacking in some degree their tissue-specific pattern, and presumably the genocopy of this, fail in the same degree to be recognized by the growth-regulating mechanisms, and thus the neoplastic state arises. It is postulated also that carcinogens induce an iso-antigenic change but that this elicits a lethal, in contrast to a non-lethal, possibly surface-active, antibody induced by the modified tissue-specific antigen. The latter type of antibody, through its prolonged action, is more likely to induce adaptation to it.

a. Nature of Promoting Agents

Cell proliferation induced by carcinogens may be a concomitant of the immune reaction induced by them. It is not produced by the non-carcinogenic TI compounds and therefore is presumably not the result of increased iso-antigenic immunity. Nor do these compounds prevent the epidermal antimitotic action of cortisone (Green & Parish, unpublished observations). It is possible, therefore, that the proliferating agent is released by an immune reaction involving the cytoplasmic lipoproteins. Lipids may well be incriminated in promoting action for we have found that certain unsaturated fatty acids and lecithin stimulate epidermal mitosis. It is perhaps significant that croton oil, rich in such acids, and certain non-ionic lipophilic-hydrophilic surface-active agents (e.g. stearates, oleates) have a powerful promoting action (Setälä, Holsti, Lundbom & Setälä, 1956). If phospholipid protein complexes are involved in the immune reaction, the release of lipids with a promoting action is a possibility. These should be cytoplasmic lipids, as the non-carcinogenic TI compounds have neither initiating nor promoting action and their action is, in theory, limited to iso-antigens probably sited in the nucleus. It is recognized that a promoting agent is not merely a stimulator of mitosis and that the process is more akin to delayed maturation (Berenblum, 1954), but this does not affect the argument that lipids are responsible for the effect.

An alternative explanation is that any deficiency of a key substance in the cytoplasm may trigger off mitosis as, for example, in virus infections. Protein-binding could induce such a deficiency and it may prove that cell proliferation induced by carcinogens, and that typical of the cancer cell, are biologically closely allied.

b. Burnet's Views on the Theory

At this stage the views of Burnet (1956) should be considered. He accepts our general concept of carcinogenesis and suggests a scheme accounting for the TI action of the non-carcinogenic hydrocarbons. According to this both the carcinogen and non-carcinogen, by combining with a marker (i.e., identity protein), produce a new pattern.

Host	Carcinogen	Inhibitor	Cancer Cell
A	A++	A+	
B B	b b	b b	b b
C C	C C	C C	C C
D D	D D	D D	D D

Both A and B components are modified, but the specific component A is lost in the carcinogen treated. The TI compound exerts its effect by inducing anti-b activity on the cancer cell, which is presumed to be a poor liberator of markers. The scheme overlooks the fact, however, that non-carcinogenic TI compounds act on homologous, and not on spontaneous, tumours. The scheme also implies that the tumour retains modified antigen, for which there is no evidence. It is based on the mistaken idea that TI compounds inhibit chemically induced tumours. It is therefore concluded that our view—that loss of identity proteins (self-markers) in itself comprises malignancy—cannot be deduced from the evidence, as, once the cancer appears, foreign proteins would no longer be produced and circulating antibody to them would have no effect. This is exactly what seems to happen, and it was one of our main arguments for antigenic loss as the cause of neoplasia (Green, 1954).

Nevertheless Burnet proceeds to support the theory, but in a short article all his ideas concerning the relation of self-markers to neoplasia cannot be discussed. There is possibly some confusion about the nature of self-markers, for they appear to be identified solely with iso-antigens. It is difficult to relate the idea of tissue organization by self-markers and corresponding recognition units to antigens common to every body cell. If, however, the identity protein (marker) molecules are postulated to be specific cytoplasmic complexes, the idea of a continuous exchange of these, coupled with an appropriate recognition mechanism, as the normal growth-regulating mechanism, is attractive. Absence of such a complex could then account both for the unco-ordinated growth

of cancer and its faulty organization and function. We have discussed other possible explanations of unrestricted growth (Green, 1958), but whichever proves true it is significant that present thinking favours the view that not only carcinogenesis, but the organization and regulation of normal tissue growth, may ultimately be explained in immunological terms.

c. The Nature of the Antigenic Deficiency

The nature of the proteins lost during carcinogenesis is uncertain but we have suggested that they are lipoprotein complexes (Green, 1957, 1958). Supporting this view is the fact that during azo-dye carcinogenesis the dye is bound to a liver fraction comprising only 8–15% of the soluble proteins and the tumour itself contains only 18% of the normal amount of this fraction (Salzberg & Griffin, 1952). Also liver lipoproteins show a progressive decline in rats injected with benzopyrene (Calcutt & Payne, 1955) and this substance binds to serum lipoproteins in vitro (Chalmers, 1955). Enhancement of tumour growth by tumour lipoprotein (Green & Wilson, 1956) gives another clue, for certain TI compounds when chemically slightly modified show enhancing, instead of inhibitory, properties. The appearance of abnormal serum lipids during carcinogenesis may be the result of lipoprotein breakdown. The chemical nature of the tumour viruses may also be relevant here, as the Rous sarcoma virus has a high phospholipid content, and it may replace a lipoprotein complex conferring identity on the cell. Antibodies to lipoproteins appear to be of the surface-active, incomplete or blocking type, capable of inducing—by adaptation or selection—a race of cells with a modified lipoprotein structure. Tumour enhancement by lipoprotein antibody may not be so far biologically removed from tumour induction by carcinogens. It is therefore postulated that the first step in chemical carcinogenesis is binding with certain specific cytoplasmic lipoproteins and that all further progression is linked up with the immune reaction thus set in motion.

The tissue-specific antigen or antigens is not an imaginary concept, but a detectable entity by immunity techniques (Feldmann & Yaffe, 1957). Weiler (1956) has shown, using fluorescein-labelled antibody, that they gradually disappear both in liver and kidney carcinogenesis. Hughes, Louis, Dineen & Spector (1957) throw doubt on this work and suggest that early necrotic changes may be responsible. However, using the plate-gel technique, which detects soluble antigens, Seligmann, Grabar & Bernard (1955) detected only two antigens in the lymphocyte of acute leukaemia, compared with three in the normal, and we have inconclusive evidence of a similar kind in human cancer. Much more direct evidence is yet required.

d. Hormonal Relationships

The hormonal aspects of tumour development are instructive, for carcinogens have hormone-mimetic actions and hormones may be tumorigenic. Now hormones, particularly of the steroid class, are tissue-specific, and their "target" proteins are presumably tissue-specific antigens. The modified hormonal action of carcinogenic hydrocarbons (see Jull, 1956) may be due to combination with such antigens, and possibly both hormonal and carcinogenic stimuli to cell division are akin. The fact that the tumour itself often shows hormonal independence strongly suggests a complete absence of a tissue-specific antigen at this stage of progression.

The tumorigenic action of excessive hormonal stimulation is due, in some instances at any rate, to a promoting action on initiated cells (Jull, 1956). In other instances it is possibly due to long-continued hyperplasia causing an excessive demand on the metabolic pool and degenerative changes in the tissues concerned. Both factors combine to produce a nutritional deficiency (as in prolonged tissue culture) which favours the outgrowth of a cell lacking a highly organized protein complex.

e. Cancer Induced by Radiation and by Viruses

No cancer theory that did not cover the tumours induced by radiation and by viruses would be complete, but we have dealt with these elsewhere (Green, 1958). Ionizing radiations produce structural, and presumably therefore antigenic, changes in protein and are thus capable theoretically of inducing the sequence of events postulated for chemical carcinogenesis. Tumour viruses may be such, because certain of their identity proteins closely resemble those of the host cell. They could then cause a loss of tissue-specific antigen by replacing it, or by competing for the same nutritional factors, or by inducing an immune reaction involving both the virus and the related identity protein and thus inducing an adaptation to a cell lacking both.

f. Aggressive Properties of Tumour

We have seen that lack of immunological identity can account for unrestrained growth. It may also account for a positive aggressive factor concerned in invasion and metastasis. The remote systemic lesions of human cancer suggest this, but the anaemia of cancer may provide evidence of it. We (Green, Wakefield & Littlewood, 1957) have shown that there may be an antibody on the surface of the red cells of some cancer patients and tumour-bearing rats, of a cold, incomplete, type. Experimentally tumours appeared to produce red-cell antibody; this suggested that they might produce antibody to host cells, given the right conditions. These would be that the parent cell was an antibody producer and that the tumour cell was lacking in an antigen present in the affected host cell. Some RE tumours theoretically meet these requirements, and such tumours are particularly prone to induce anaemia. It was postulated that malignant cells generally might attack appropriate host cells because the antigen deficiency was a post-natal change to which no acquired tolerance had developed. Rapid loss of weight, fever and remote organic lesions might thus be explained. Spread of tumours might be in part due to active aggression and cell infiltrations around them, expressions of an immune reaction.

g. Resistance of Host to Tumours

The states of dormancy and progression suggest that there is also an active resistance on the part of the host. We have suggested (Green, 1958) that RE scavenging of displaced cells may be weaker for the cancer cell since it contains less of the modified identity proteins by which it is recognized. But the normal disposal mechanism may still be able to keep metastatic spread under control. Metastatic cells, if not rapidly removed, may possibly, in an abnormal environment, undergo some qualitative change in identity protein which elicits an immune reaction in the host, causing further antigenic loss and hence increased malignancy. The processes of carcinogenesis, tumour spread and progression may all prove to have a common immunological basis.

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ASPECTS OF ENDOCRINE CARCINOGENESIS

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During the last decade views on the role of hormones in carcinogenesis have undergone considerable modifications following progress in endocrinology. Not only have new hormones been recognized and isolated and the metabolic transformations of many steroid hormones clarified, but also knowledge of the mechanisms which regulate the secretion of these agents has been widened with the result that the pituitary, once called the master gland, is now considered by many to be the servant of the hypothalamus (Harris, 1955). The student of neoplastic diseases has been able to make use of numerous recent advances. To quote a few examples, we may mention the concept of Ingle (1954) of the permissive role in hormones, the interdependence of hormonal activity, and the realization that not only the gonads, but also other endocrine glands like the adrenal, are under the control of more than one trophic hormone of the adenohypophysis. The cancer research worker, on his part, could supply the endocrinologist or biochemist with valuable tools by way of experimentally induced or spontaneously occurring functional tumours of endocrine glands.

1. Mechanisms of Hormone Action in Neoplasia

a. Direct Action of Hormones

The primary action of hormones has long been a matter for speculation and it is difficult to interpret the physiological effects of hormones by the observed changes in enzyme patterns. Now a new hypothesis is gaining ground. The work of Levine & Goldstein (1955) strongly suggests that insulin acts by altering the permeability of muscle cells and facilitates transfer of sugar from extra- to intra-cellular space without influencing enzymic mechanisms. Likewise the studies of Hechter (1957) revealed striking changes in the structures surrounding adrenocortical cells under the influence of ACTH.

At present the student of endocrine pathology can take into account only relatively late effects of hormone action as they reveal themselves by gross changes in form and function of whole organs or some of their specialized cells. When he speaks of direct action, he means either stimulation or inhibition of target organs, as for instance stimulation of the thyroid by thyrotrophic hormone, or suppression of the FSH-producing cells by oestrogens. Indirect action is more difficult to define. Sometimes the term is used to indicate a chain reaction: stimulation of uterus and vagina after injection of gonadotrophins would be a case in point. Sometimes, however, the term implies that an observed effect is obtainable only in the intact animal but not after ablation of an endocrine gland which is not the target organ of the hormone under investigation. For example, Moon, Simpson, Li & Evans (1950a, 1950b, 1950c, 1951) induced tumours of various organs in rats by prolonged treatment with growth hormone. Hypophysectomized animals failed to respond in a similar manner.

When a target organ is stimulated by the appropriate hormone, the first reaction is hyperaemia, followed by increase in weight, and also by hyperplasia. Often this is the only result obtainable even if the stimulus is maintained during the entire life-span of the animal. In other instances the diffuse type of tissue growth is followed by nodular hyperplasia, and finally structures bearing the characteristic features of neoplasia appear in the target organ. The study of the experimental tumours of the thyroid and ovary has provided satisfactory evidence that this sequence of events occurs and is due to direct hormonal action.

b. Permissive Action of Hormones

Chemical carcinogenesis in the liver is dependent on pituitary and/or adrenal hormones. Hypophysectomy prevents induction of hepatomata by 3′-methyl-4-dimethylaminoazobenzene (Griffin, Rinfret & Corsigilia, 1953) and by diacetylaminofluorene (O'Neal & Griffin, 1957) which, however, retains its carcinogenic activity for other tissues. Therefore failure to transform the aromatic amine into the active compound can be ruled out. The liver of the hypophysectomized rat still binds the azo dye, but signs of liver damage are missing. The usual reaction to it is restored by administration of either ACTH or growth hormone (Griffin, Richardson, Robertson, O'Neal & Spain, 1955). In *New pathways in*

cellular pathology, Cameron (1956) mentions that thioacetamide, another liver carcinogen, is ineffective in adrenalectomized animals. Bielschowsky & Hall (1953) observed that thyroidectomized male rats treated with 2-aminofluorene (AF) failed to develop hepatomata, whereas in other organs cancers were found. After complete ablation of the thyroid the pituitaries are void of acidophils. Injection of growth hormone into such animals re-established the susceptibility of the liver to AF (Bielschowsky, 1956). Thus lack of thyroxin cannot be responsible for the refractory state of the liver. Hepatoma formation can take place only when at least limited amounts of growth hormone are present. The livers of partially thyroidectomized rats, having greatly reduced numbers of acidophils in the pituitary, respond to AF nearly as readily as those of intact animals. These observations point to a permissive role of growth hormone in the pathogenesis of hepatomata.

2. Definition of an Endocrine Tumour

Endocrine carcinogenesis became a major aspect of cancer research after Lacassagne (1932) first demonstrated that the naturally occurring steroid hormone oestrone was implicated in the cause of breast cancer in mice. This led gradually to general recognition that hormones are significant factors not only in the aetiology, but also in the prevention and treatment of cancer.

There are, broadly speaking, two different types of cancer which occur in animals and man. To describe these tumour-types the terms "hormone-dependent" and "hormone-independent" were first used by Huggins & Scott (1945) to denote those neoplasms which depend upon hormones for their maintenance or those which grow independently of them.

a. Hormone-Dependent Tumours

The growth of the former type of malignant tumours can be temporarily controlled by an alteration of the hormonal environment in which they develop. Dependent growths are often composed of cells which have been stimulated to proliferate because of a hormonal imbalance, but in some instances they can be induced by extrinsic agents such as irradiations, carcinogenic hydrocarbons, or hormones in combination with irradiation.

b. Hormone-Independent Tumours

Autonomous or hormone-independent lesions, on the other hand, such as those which develop in the skin, stomach and lung, are not under direct endocrine control, nor can their growth or behaviour be influenced by any form of endocrine modification. The dividing line between these two basically different types of cancer is not, however, absolute, as a certain number of hormone-dependent tumours give rise to autonomous variants.

When a tumour becomes autonomous it is composed of permanently altered cells which do not respond any more to the hormonal forces controlling the cells from which the tumour originated. It is conceivable that some malignant growths might be composed of both dependent and autonomous cellular components. This might explain why a hormonal-dependent tumour, whose growth had been previously held in check by means of endocrine therapy, will suddenly lose its responsiveness to a particular hormone or combinations of hormones and become an uncontrolled autonomous neoplasm.

3. Tumours of the Pituitary

Histologists distinguish three types of cells in the adenohypophysis: acidophils, basophils and chromophobes, and the pituitary tumours have been classified accordingly. In recent publications two contradictory points of view have been put forward. Some authors are of the opinion that a particular identifiable cell can secrete a variety of pituitary hormones (Russfield, 1955); others believe in the existence of specialized, monofunctional cell types (Purves & Griesbach, 1951, 1954; Herlant, 1956). In our opinion undifferentiated non-functional cells exist in the anterior lobe but, once differentiation has taken place, the resulting mature cell types are committed to manufacture one specific product. All chromophilic elements belong to this class. By comparison of pituitary hormone content and state of target organs with the cytological picture as revealed by modern staining techniques, it was possible to localize some pituitary hormones in specific cells: growth hormone and prolactin in two types of acidophils, thyrotrophic and gonadotrophic hormones in three types of basophils. In this paper the pituitary tumours have been classified according to their function because stainability varies with the state of secretory activity.

Recent experimental work on pituitary changes in neoplasia is discussed in the stimulating booklet by Lipschütz (1957).

a. Somatotrophic Tumours

Somatotrophic tumours are rare in laboratory animals. Apart from a transplantable autonomous neoplasm which, according to Furth (1955), had weak thyrotrophic and marked growth-promoting properties, only one other tumour of this type has been recorded. Schlumberger (1956) found an adenoma in a parakeet of which the grafts secreted growth hormone and induced obesity.

b. Mammotrophic (Prolactin-Secreting) Tumours

Cramer & Horning (1936) were the first to obtain neoplastic lesions in the pituitary by the administration of oestrogen. Zeckwer (1944) and Lacour (1950) suggested that increased amounts of prolactin were secreted by such tumours. Direct stimulation of the breast by oestrogen could be excluded in rats found at autopsy to have hyperplastic and secreting mammary glands in conjunction with an acidophilic pituitary adenoma, whereas uterus and vagina were atrophic (Bielschowsky, 1954).

That the mammotrophic effect of the oestrogen-induced adenomata is due to prolactin was firmly established by hormone assay (Meyer & Clifton, 1956) and by the work of Furth and his collaborators (Furth, Gadsden, Clifton & Anderson, 1956; Furth, Clifton, Gadsden & Buffett, 1956) on transplantable mammotrophic tumours of mice and rats. The mouse tumours originated in old females which had been exposed to whole body irradiation. They were responsive to oestrogen and grew slowly in normal animals. The rat tumours obtained by implantation of stilboestrol pellets could be propagated only in oestrogen-treated hosts but became autonomous in later transfers. Both types of graft stimulated the mammary glands of female recipients. Mühlbock (1953a) described spontaneous, prolactin-secreting adenomata in mice, and Bielschowsky, Bielschowsky & Lindsay (1956) studied the histogenesis of such neoplasms in the NZY strain of mice, in which 80% of intact females had enlarged pituitaries. Ovariectomy prevented hyperplasia and subsequent

tumour formation in the adenohypophysis except in those animals in which oestrogen-secreting adrenocortical tumours developed (Bielschowsky, 1956, 1958). None of the primary pituitary tumours observed in NZY mice produced the somatotrophic effects so often observed by Furth and his colleagues (Furth, Gadsden, Clifton & Anderson, 1956; Furth, Clifton, Gadsden & Buffett, 1956) in mice and rats bearing transplanted mammotrophic tumours. The development of this type of pituitary adenoma (see Plate I, fig. A) depends on a genetically determined susceptibility of the prolactin-secreting cells to oestrogen (Gardner, 1953). In the early stage of their development, withdrawal of the stimulus leads to regression (Nelson, 1944; Bielschowsky, 1958).

Hypothyroidism seems to inhibit the action of oestrogens on the pituitary, and hyperthyroidism to enhance it (Gillman & Gilbert, 1955). In contrast to rats or mice, hamsters react to oestrogens with tumorous enlargement of the intermediate lobe (Vazquez-Lopez, 1944). These neoplasms seem to interfere with melanogenesis in the skin (Horning, 1958).

c. Thyrotrophic (TSH-Secreting) Tumours

Thyrotrophic tumours of the pituitary have been found in mice and rats suffering from chronic thyroid hormone deficiency. Ablation of the thyroid (Furth, Dent, Burnett & Gadsden, 1955), destruction of the gland by ^{131}I (Gorbman, 1949), inhibition of thyroxin synthesis by goitrogens (Seifter, Ehrich & Hudyma, 1949), an inadequate iodine content of the diet (Bielschowsky, 1953) all lower the level of thyroxin and induce hyperplasia of the thyrotrophs. Halmi & Gude (1954) have given an excellent account of the changes occurring in the adenohypophysis of the mouse after destruction of the thyroid by treatment with ^{131}I, and Furth (1954) described the effects of TSH-secreting tumours and their transplants on the thyroid and other organs. At first the grafts grew only in athyroid mice, but later generations could be propagated in normal animals. The dependent as well as the autonomous transplants metastasized.

Is it hypersecretion by a hypothalamic centre which triggers off hyperplasia of thyrotrophs whenever the concentration of thyroxin falls below a critical level; or does thyroxin cease, under these circumstances, to be an effective regulator of thyrotrophs? At present one can only point out that dependent tumours grow progressively at sites remote from the hypothalamus and that thyroxin inhibits equally well the proliferation of dependent tumour cells and that of normal thyrotrophs. Whatever the mechanism, if the hormonal imbalance which leads to increase in numbers and in secretory activity of thyrotrophs is not redressed, a process is set in motion which leads to an autonomous neoplasm. Furth (1957) has stressed that failure of a feed-back mechanism is the decisive factor in the pathogenesis of thyrotrophic tumours. One of us (Bielschowsky, 1955a) has emphasized that they are an attempt to compensate for thyroxin deficiency. Both points of view are compatible.

d. Gonadotrophic (ICSH-Secreting) Tumours

Histological study of pituitaries of rats with experimentally induced cryptorchidism (Bielschowsky, 1955b) revealed, apart from a general increase of gonadotrophs, the presence of nodules composed of cells closely resembling the LH cells of Purves & Griesbach (1954). Progression to adenomata was seen (see Plate I, fig. B). There was a good correlation between numbers of central gonadotrophs (the ICSH cells) and the degree of hyperplasia of Leydig cells; of all the target organs of the pituitary they alone were found invariably stimulated. The pituitary reacts to testicular failure in a similar manner as it does to gonadectomy. Whether complete lack of sex hormones is more prone than subnormal amounts to induce gonadotrophic tumours (Griesbach & Purves, 1956) remains to be investigated.

The basophilic tumours of the pituitary described in mice (Dickie & Woolley, 1949; Dickie & Lane, 1956) and in rats (Houssay, Cardeza & Pinto, 1955) with post-castrational adrenocortical lesions secreting sex hormone may well be gonadotrophic adenomata.

e. Adrenotrophic (ACTH-Secreting) Tumours

ACTH-secreting tumours of the pituitary were discovered by Furth (1955) in mice exposed to whole body irradiation. They could be transplanted into normal animals in which the following signs developed: lymphopenia and eosinopenia, obesity, polyuria, moderate hyperplasia of all three layers of the adrenal cortex, atrophy of thymus and other lymphoid tissue, and lowered resistance to generalized infections. Adrenalectomy reversed these changes and speeded up the growth-rate of tumour grafts.

f. Bio-Assays of Transplantable Pituitary Tumours

After several passages one of Furth's lines of adrenotrophic tumours was assayed for ACTH and melanocyte-stimulating hormone (MSH). ACTH was present in low concentration, but MSH at a level equal to that found in normal pituitaries (Steelman, Kelly, Norgello & Weber, 1956). In view of the close chemical relationship between the two hormones this result does not invalidate Furth's conclusions as to the function of the original tumour.

Bates, Clifton & Anderson (1956) determined the prolactin and TSH content of various transplantable pituitary adenomata. The adrenotrophic tumours contained neither hormone; the thyrotrophic tumours were free of prolactin but rich in TSH. The mammotrophic tumours contained little or no TSH, and the concentration of prolactin varied according to the source of the material tested; the hormone-dependent tumours contained ten times the amount of that found in autonomous tumours.

Transplantation has been used successfully for the study of functional tumours. It should, however, be remembered that, as shown by Haddow, Horning & Smith (1957), transplants of non-functional neoplasms affect profoundly the endocrine system of the host. Also, during serial transplantation, many functional neoplasms change to an extent that makes it impossible to use them as indicators of the properties of the original neoplasm.

4. Tumours of the Ovary

Ovarian neoplasia can be readily produced in laboratory rodents by widely different procedures. Whether caused by irradiation, by ovarian grafting, by hormones or by chemicals, ovarian tumours appear to arise from a disturbance of the normal pituitary-ovarian relationship. Irrespective of the method by which they are induced, many of these tumours are hormone-dependent for their sustained growth when transplanted into host animals. Thus they constitute excellent examples of endocrine neoplasia.

It is of interest to note that they rarely occur spontaneously

ASPECTS OF ENDOCRINE CARCINOGENESIS

(FIGS. A–H)

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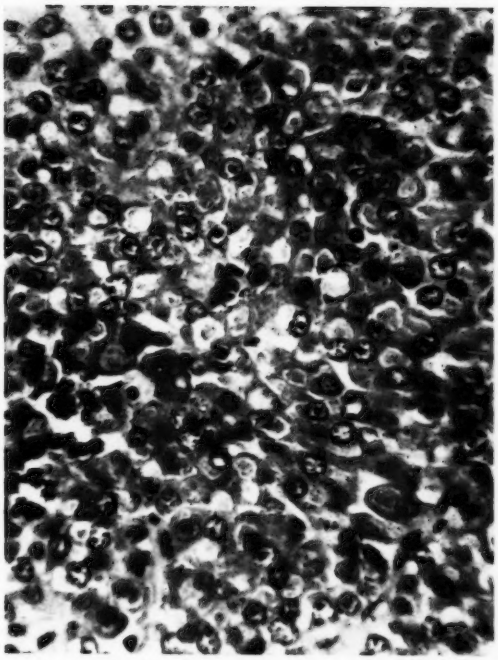

FIG. A. Mammotrophic (acidophilic) tumour of pituitary from rat with oestrogen-secreting granulosa-cell tumour. Note varying intensity of acidophilic granulation

(Stain: Green-Papanicolaou; magnification × 450)

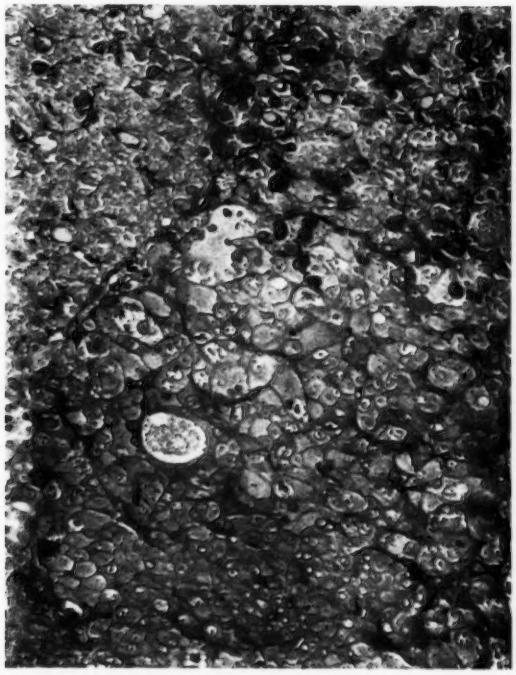

FIG. B. Gonadotrophic (basophilic) tumour of pituitary from rat with ectopic testis. Majority of large tumour cells give only weak periodic acid-Schiff (PAS) reaction in contrast to the normal gonadotrophic cells outside the adenoma

(Stain: PAS and haematoxylin; magnification × 150)

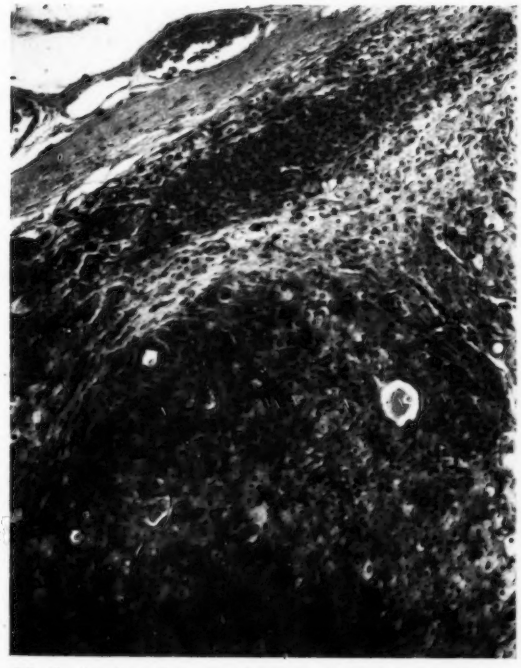

FIG. C. Leydig-cell tumour from rat with ectopic testis

(Stain: Haematoxylin and eosin; magnification × 150)

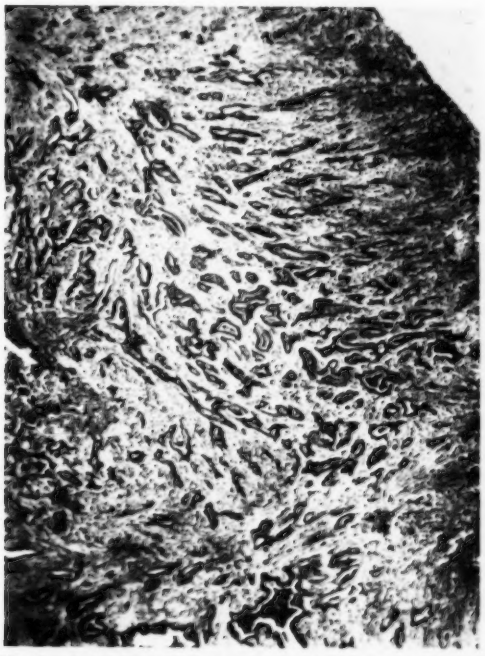

FIG. D. Endometrial adenocarcinoma from rat treated with stilboestrol (0.5 mg.) for 21 months

(Stain: Haematoxylin and eosin; magnification × 40)

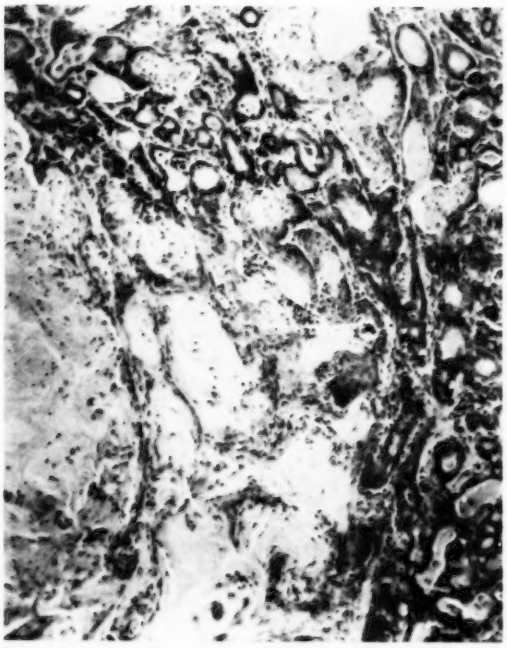

FIG. E. Spontaneous adenoacanthoma in NZY breeder aged 18 months

(Stain: Haematoxylin and eosin; magnification × 150)

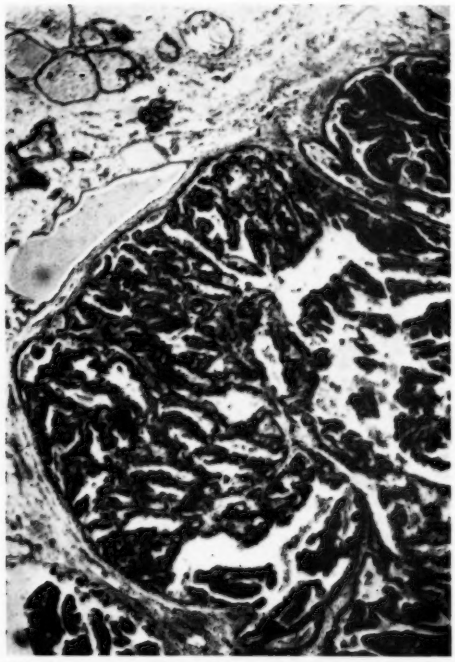

FIG. F. Intraductal papilloma induced in male rat by stilboestrol

(Stain: Haematoxylin and eosin; magnification × 40)

FIG. G. Bilateral and multifocal kidney tumour in a male golden hamster, induced by stilboestrol

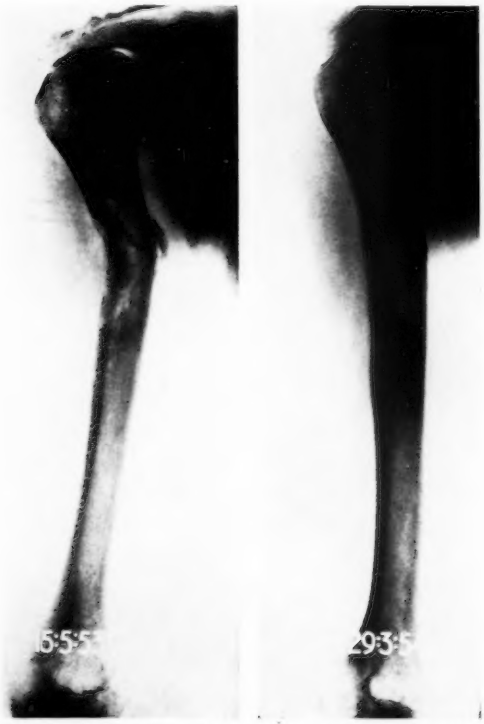

FIG. H. Two *x*-ray photographs taken of a woman patient with a metastasizing carcinoma of the breast. The left-hand picture reveals a secondary lesion in the humerus with a resulting pathological fracture. The right-hand picture, taken several months after bilateral adrenalectomy, shows a recalcification of the bone and a disappearance of the growth. (With kind permission of Sir Stanford Cade)

in mice (Gardner, 1953). Slye, Holmes & Wells (1920) found only 44 cases of ovarian cancer among 22,000 mice, 38 of which were described as benign lesions and only four as primary malignant growths. Spontaneous ovarian tumours in the rat have been described by Bullock & Curtis (1930), and by Iglesias, Sternberg & Segaloff (1950), but the incidence of these tumours in this species is very low.

a. Tumours Induced by a Gonadal-Pituitary Imbalance Alone and in Combination with Irradiation

The technique of parabiosis, by which two or more animals of the same genetical constitution are sutured side by side so that they possess a common blood supply, has rendered valuable information on the relationship existing between the gonads and the gonadotrophic functions of the hypophysis. When a normal female is joined in parabiotic union with either a castrated male or female, the gonadotrophic hormone secreted in excess by the castrate is transferred to the intact partner and stimulates its ovaries. Although more oestrogen is produced, it is rapidly broken down by the liver and consequently does not pass over to the castrate whose pituitary gland remains uninhibited. An account of the hormonal mechanism, together with the early histological changes induced by the ovary in the rat, was described in detail by Zeckwer (1944). This method has been used successfully by Mühlbock (1953b, 1954; van Nie & Mühlbock, 1956), to induce granulosa-cell tumours of the ovary in mice. Earlier Bielschowsky & Hall (1951) had found that the induction of ovarian tumours could be accelerated in intact female rats treated with 2-acetamidofluorene (AAF) and parabiosed to gonadectomized litter mates. Ovarian lesions developed in over 50% of these animals, many of which were of the granulosa-cell type. Because the liver of rodents inactivates ovarian steroids so efficiently, tumours can be readily induced in ovaries grafted into the spleen of gonadectomized hosts (Biskind & Biskind, 1944). The oestrogens produced by the transplanted ovary enter the liver via the splenic vein and are inactivated before entering the general circulation. Consequently the pituitaries of the rodent carrying the intrasplenic graft are of the castrate type and possess an elevated gonadotrophin content. These are interesting examples of how neoplasia can arise by artificially creating a hormonal imbalance which eventually leads to an excessive production of gonadotrophins, which in turn induces ovarian cancer.

The age factor appears to play a role in the induction of ovarian tumours by this technique. Smith & Gardner (1950) found that few tumours develop when old ovaries are transplanted into the spleens of old hosts but, when old ovaries are implanted into young mice, tumours developed to the same extent as in young ovaries transplanted into young hosts. They contend that this is because pituitary gonadotrophins decrease in rodents with advancing age. Sex is also another factor involved in the production of ovarian neoplasia in mice. Li & Gardner (1947) found that in gonadectomized rodents granulosa-cell tumours develop predominantly from intrasplenic grafts in males and that mixed granulosa-cell lesions and luteomata are more frequent in female mice.

Irradiation of intrasplenic ovarian grafts, according to Li, Gardner & Kaplan (1947), does not affect the incidence of ovarian tumours nor reduce the latent period of induction. Mühlbock (1953a) showed that the latent period for the induction of tumours from ovarian intrasplenic grafts can be considerably reduced by parabiotic union between the experimental and castrated animal. Li & Gardner (1947) have, however, found that tumours do not develop from intrasplenic ovarian grafts when the castrated host is treated with either oestrogens or testosterone. In the same series of experiments they report that daily injections of a gonadotrophic preparation have no inhibitory effect on ovarian tumour development. As the induction of ovarian tumours by this method is due to a change in the balance between gonadotrophic and ovarian hormones, these results of Gardner and Li are of interest. Another method of inducing ovarian neoplasia in rodents is by röntgen irradiation. Drips & Ford (1932) were the first to observe early neoplastic changes in the ovaries of irradiated rats. Later Furth & Butterworth (1936), whilst studying the effects of irradiation on mice with leukaemia, discovered that many of these mice later developed ovarian tumours, most of which were of the granulosa-cell type.

After numerous investigators had confirmed the induction of ovarian tumours by irradiation, attention was focused on the underlying hormonal mechanism. Lorenz, Heston, Eschenbrenner & Deringer (1947) found that the induction of malignant growths by röntgen irradiation is dependent upon a minimum rather than upon a total dosage. If given as a single dose, 25–50 r. appears to be the minimum for the induction of ovarian neoplasia in mice. This is of interest as Furth (1949) and Gardner (1953) contend that the hormonal imbalance responsible for ovarian tumorigenesis is due to the fact that irradiation reduces oestrogen production, and that this diminished output allows the pituitary gland to increase production of gonadotrophins. This effect was further substantiated by Lick, Kirschbaum & Miscer (1949) who found that tumour formation did not occur following irradiation of a single ovary, if the second ovary was removed before *x*-ray treatment.

b. Chemically Induced Ovarian Tumours

There appears to be little doubt that the techniques so far discussed for inducing ovarian tumours in rats and mice are caused by an endocrine imbalance which finally results in tumorigenesis. It is, however, more difficult to attribute the hormonal theory of ovarian tumour induction to those lesions produced by treatment with carcinogenic hydrocarbons alone. A possible explanation might be that the carcinogens produce a state of anoestrus which would probably cause an increase of pituitary gonadotrophins, which in turn would stimulate the ovary.

The first workers to describe ovarian tumours induced in mice by treatment with a chemical compound alone were Engelbreth-Holm & Lefèvre (1941) who observed these lesions in mice treated with 9:10-dimethyl-1:2-benzanthracene (DMBA). Later Howell, Marchant & Orr (1954), using the same compound, obtained 53 ovarian tumours in 88 female mice, all of which were painted with the compound at fortnightly intervals. Most of these were granulosa-cell tumours; a few luteomata also developed. Over the same period of time these authors treated another series of mice with 20-methylcholanthrene (MC) alone, but failed to obtain tumours. Earlier Furth & Boon (1947) had reported a luteoma in a mouse which had been painted with MC and treated with *x* rays. It grew when grafted in both male and female mice, and appeared to secrete an androgenic hormone. In another series of experiments Furth & Sobel (1947) found that only two ovarian tumours occurred in 134 mice treated with the carcinogen alone. They state that ovarian lesions

appeared at an earlier age in mice which received the combined treatment of *x* rays and MC than in those which received the *x* rays alone. The high incidence of ovarian tumours in mice treated with DMBA, compared with the low incidence obtained by treatment with MC, is an interesting example of the tissue specificity possessed by these carcinogenic hydrocarbons.

5. Tumours of the Testis

The literature on the oestrogen-induced tumours of the testis has been reviewed by Burrows (Burrows & Horning, 1952, p. 59), one of the pioneers in this field. Recently Andervont, Shimkin & Canter (1957) found that the majority of early Leydig-cell nodules induced by stilboestrol persisted when the pellet was removed, but needed further stimulation by oestrogen for progression to macroscopically recognizable tumours. Transplantation studies revealed that the removal of the pellet at an early stage of the experiment aided the development of independent growths, i.e., of tumours which could be propagated in mice not treated with oestrogens. Clifton, Bloch, Upton & Furth (1956) studied the effects of transplanted Leydig-cell tumours on the hosts. Apart from androgenic effects, the grafts produced atrophy of the adrenal cortex. Female mice bearing transplants of a spontaneous Leydig-cell tumour had large uteri with cystic hyperplasia of glands. Deciduomata were seen in these mice, an indication of high progestational activity of the grafts. Gardner (1958) showed that it is the susceptibility of the interstitial cell which determines the response to oestrogen in different strains of mice. Oestrogenic stimulation of a hybrid led to tumour formation in transplanted testes only in donors of susceptible strains. From other experiments it is obvious that stimulation of Leydig cells by gonadotrophin is essential. Thus Biskind & Biskind (1945), Twombly, Meisel & Stout (1949) and Jones (1955) obtained Leydig-cell tumours in castrated rats bearing testicular implants in the spleen. The same applies to the neoplasms arising in cryptorchid testes of mice (Huseby, 1958) and of rats (Bielschowsky & Hall, 1954) (see Plate I, fig. C). Of 57 rats which survived the operation for 14–24 months: two developed Leydig-cell tumours; four, basophilic adenomata of the pituitary; and four, fibroadenomata of the breast; nodular hyperplasia of Leydig cells was frequent. None of these lesions has ever been observed in untreated males of the Dunedin colony.

One of the three spontaneous Leydig-cell tumours described by Guérin (1954) occurred in a rat in which a mammary fibroadenoma, an adenoma of the pituitary, and unilateral hyperplasia of the thyroid, were also present. Gillman, Gilbert & Spence (1953) found spontaneous Leydig-cell tumours in 20% of their rats, a unique observation.

That gonadotrophic stimulation aids teratoma formation in testes of cocks has been confirmed (Smith & Powell, 1957).

6. Tumours of the Kidney, Epididymis, Bone; Leukaemia and Lymphoma

a. Renal Lesions, Including Neoplasia (in Hamster), Caused by Oestrogen

Apart from the occasional production of urinary calculi and glomerulonephritis, oestrogen administration has no marked effect on the kidneys of rats and mice (Pfeiffer, Emmel & Gardner, 1940; Korenchevsky & Ross, 1940). Recently Chesterman, Franks, Knudsen & Williams (1956) observed more pronounced pathological renal lesions in guinea-pigs, following prolonged treatment with stilboestrol. There is, however, no record that oestrogen treatment induces kidney tumours in rodents, with the exception of hamsters. Matthews, Kirkman & Bacon (1947) first demonstrated the peculiar susceptibility of the golden hamster to renal neoplasia, following the administration of oestrogen. These observations were confirmed by Horning & Whittick (1954) who also described the histogenesis of these kidney lesions, and by Ising (1956).

Kidney tumours in hamsters treated with oestrogen are sex limited. They develop in approximately 90% of males (see Plate II, fig. G) but not in intact females. Kirkman (1957) has, however, obtained tumours in gonadectomized females.

Occasionally multiple primary tumours arise in other organs of the hamster, which indicates their sensitivity to oestrogenic stimulation. Horning (unpublished work, 1954) reported a low incidence of renal neoplasia in hamsters treated with 3:4-benzopyrene. This suggested that kidney cancer in the hamster might in part be due to absorbed chemical carcinogens acting selectively on the renal epithelium during excretion. It is of interest to note that this hydrocarbon also possesses oestrogenic activity (Cook & Dodds, 1933).

Transplantation experiments demonstrate that renal neoplasia in hamsters are hormonal cancers. These kidney tumours are dependent upon excessive oestrogen stimulation not only for their induction, but also for their sustained growth as transplants in host animals. These tumours have been growing as serial grafts in hamsters for over four and a half years in the Chester Beatty Research Institute and are still hormone-dependent lesions, since they fail to grow in normal untreated hosts (Horning, 1956).

The induction of renal carcinoma in the hamster is of special interest in the problem of endocrine carcinogenesis. Tumours which are induced by hormones and which are dependent upon them for continuous growth generally develop in organs which belong to the endocrine system, and come under the direct influence of the anterior hypophysis. The kidney, except as a part of the body subject to the stimulation of somatotrophin, is not directly influenced by the pars anterior. Thus these oestrogen-induced lesions constitute an anomaly in endocrine cancer.

b. Metaplasia and Tumours of the Epididymis

Excessive oestrogenic stimulation causes atrophy of the epididymal epithelium in mice and rats, which is accompanied by a loss of its secretory activity and an increase in the fibromuscular stroma. In some instances oestrogens induce a squamous metaplasia in the epididymis of the mouse but not in the rat (Burrows & Horning, 1952, p. 75). Vazquez-Lopez (1944) was the first to report cancer of the epididymis in the hamster by treatment with oestradiol benzoate alone. The lesions were bilateral, with metastases. More recently Bacon (1952) has induced primary tumours of the epididymis in approximately 100% of hamsters, following simultaneous treatment with diethylstilboestrol and testosterone. These lesions are transplantable provided that the host animals receive a combined treatment of oestrogen and androgen, but grafts fail to grow when implanted into hosts which have been treated with the male or the female sex hormone alone.

c. Bone Tumours

Although oestrogen alone does not induce malignant bone neoplasia, it is capable under certain conditions of influencing the formation of bone lesions; this effect is reversible by simultaneous treatment with androgen. The response, however, is not the same in all strains of mice.

The fact that a relationship has long been known to exist between the gonads and the skeleton, led to intense investigation on the effects of sex hormones on the bones of rodents. Folley (1936) and Riddle & Dotti (1936) were the first to observe the influence of oestrogen on calcium metabolism. This was confirmed by Wentworth, Smith & Gardner (1940) who showed an increase in the inorganic content of the bones of mice following treatment with oestradiol benzoate. Later Gardner (1943) found that the breaking strength of bones in mice was increased by 50%, following oestrogen treatment. This effect could be prevented by androgen administration. Oestrogenic neoplasia is said to occur in the calvarium in rodents (Sutro, 1940), but a normal osseous texture will be regained if the supply of oestrogen is stopped. Other investigators have demonstrated that androgen administration in rodents prevents the osseous changes induced by oestrogen (Burrows & Horning, 1952, p. 76).

Pybus & Miller (1938) established an inbred strain of mice with a high incidence of spontaneous bone tumours which was greater in females (77.3%) than in males (29.6%). Although the incidence in males was not increased by oestrone, the latent period of tumour development was reduced. Later Miller, Orr & Pybus (1943) showed that ovariectomy at an early age delayed the appearance of palpable tumours by as much as four months. In the males of the same strain, massive implanted doses of oestrone intensified osteoclastic activity. Palpable benign bone lesions were produced, which regressed after removal of the hormone pellets. The special strain of mice established by Miller and Pybus, in which a high percentage of females developed osteogenic sarcoma, suddenly failed to develop further tumours for reasons unknown. Spontaneous osteogenic sarcoma in mice of other strains is rare, and further studies are at present impeded by lack of suitable experimental material.

d. Leukaemia and Lymphomata

The basic character of these conditions is a partial or complete independence of the affected leucocytic or lymphoid tissue from any regulation of its growth and differentiation. The term lymphoma will be used to cover lymphosarcoma or lymphatic leukaemia. Apparently in any individual case the disease seldom affects more than one kind of cell. Although heredity and various hormones are factors influencing the condition, a viral agent may be involved in the transmission of leucosis, at least in a certain strain of mice (Gross, 1957) and in birds (Ellermann, 1921). The fact that some inbred strains of mice have a high incidence of leucosis, whereas other strains have a low one, is proof of an inherited influence (Furth, Cole & Boon, 1942; Law, 1944; Furth, 1946). The observations of Mercier (1938) that lymphomata occur more frequently in female mice than in males led to experiments by other workers elucidating the role played by hormones in the induction and inhibition of this disease. Lacassagne (1938) and Gardner, Dougherty & Williams (1944) were the first to report the occurrence of lymphomata in susceptible strains of mice, following oestrogen administration. The latter authors also found that combined treatment with androgen inhibited the development of lymphoid tumours.

After numerous investigators confirmed that exogenous oestrogens either induce or increase the incidence of lymphoma, and that androgens possess an inhibitory action, experiments were undertaken to determine the role of intrinsic hormones. Thus McEndy, Boon & Furth (1944) found that the incidence of spontaneous leukaemia in mice was reduced from 74% to 45% following ovariectomy. Murphy (1944) also reported that the incidence of leukaemia in his male mice was raised from 53% to 97% by castration. In mice of two stocks Dmochowski & Horning (1940) found that castration before puberty, followed by treatment with oestrogens, raised the incidence of lymphoma to 59% and 70% respectively. The effect of either oestrogen administration or gonadectomy on the incidence of leucosis depends to some extent on strain differences.

Prolonged administration of somatotrophin to intact rats (Long-Evans strain), according to Moon *et al.* (1950a), induces lymphosarcoma of the lung and lymph nodes. Rawson & Rall (1955) report that Nagareda and Kaplan, using the same hormone, observed a proliferation of thymic and lymphoid tissue in mice. Dougherty (1955) has also reported in a private communication that under certain conditions thyrotrophin stimulates the growth of lymphoid tissues in intact mice. According to Rawson & Rall (1955), it is possible that this effect may account for the significant occurrence of lymphosarcoma in patients suffering from long-standing Graves' disease.

After it was found that adrenocortical hormones administered to mice induce regression of lymphosarcomata (Heilman & Kendall, 1944), treatment with these hormones was first used by Pearson & Eliel (1950) on patients suffering with lymphatic leukaemia or lymphosarcoma. They found that either cortisone or ACTH caused pronounced atrophy of the tumour mass, with metabolic changes generally associated with the destruction of malignant tissues. As long as the treatment was continued, remissions of up to two years were obtained. These workers further report that patients with acute leukaemia respond dramatically to treatment with ACTH or the 11-oxygenated steroids of the adrenal, but in children the disease is more resistant (Pearson, Eliel, Talbot, Burchenal, Petro, Poppell & Craner, 1950). The fact that certain forms of leucosis can be induced and controlled by hormonal administration in both animals and man is encouraging, and indicates that the endocrinological approach to this problem should be vigorously pursued.

7. Tumours of the Adrenal

a. Tumours of the Adrenal Cortex

The neoplasms which arise after gonadectomy in the hyperplastic adrenal cortex of various strains of mice and rats (Woolley, 1950; Houssay *et al.* 1955) secrete androgenic or oestrogenic steroids. These functional tumours are a manifestation of a more or less successful attempt to compensate for the loss of ovaries or testes. They originate in animals in which high levels of gonadotrophins are maintained for many months, and all the evidence available indicates that ACTH plays no role in their development. Similar neoplastic lesions have been found in aged mice (Kirschbaum, Frantz & Williams, 1946).

Recently new types of tumours have come to hand. Franks & Chesterman (1956) found, in hamsters treated with stilboestrol, hyperplasia and adenomata of the adrenal cortex in conjunction with cardiac and renal lesions typical of severe potassium deficiency; the authors draw attention to the similarity with Conn's syndrome (hyperaldosteronism).

Starting from apparently benign cortical adenomata of uncertain aetiology, Cohen, Furth & Buffett (1957) established lines of transplantable tumours with glucocorticoid and mineralocorticoid activity. In-vitro tissue from such grafts responded to stimulation by ACTH with an increased production of ketosteroids of which two—corticosterone and 11β-hydroxy-Δ^4-androstene-3,17-dione—were identified (Cohen, Bloch & Celozzi, 1957).

b. Tumours of the Adrenal Medulla

Ganglioneuromata (Haddow & Horning, 1950; Guérin, 1954) are extremely rare in rodents. More common are phaeochromocytomata, which occur in aged rats of both sexes with a frequency which varies greatly in different colonies. Gillman *et al.* (1953), who have given the most detailed account of these neoplasms, found phaeochromocytomata in 50% of females and in 80% of males 13–18 months of age, an incidence far in excess of that recorded by others. Few phaeochromocytomata have been described in mice. Smith, Gardner, Li & Kaplan (1949) found four large and five smaller tumours in the medulla of castrated or irradiated mice belonging to different strains.

A hormonal imbalance might well be of aetiological importance. Moon *et al.* (1950b) observed marked hyperplasia of the medulla and one phaeochromocytoma in female rats treated with growth hormone, and Gillman's rats had many neoplasms in other endocrine glands.

8. Tumours of the Prostate

a. Dependence of the Prostate on Androgen

Since John Hunter (1786) studied the effect of castration on the prostate, investigations have established the dependence of the prostatic epithelium on androgen secretion. In this review only a few outstanding experiments will be discussed, such as those of Moore & Melchionna (1937) and Moore & Smith (1937) who, by grafting fragments of prostatic epithelium into the anterior chamber of the rabbit eye, were able to record fluctuations in size of the grafts photographically. A rapid diminution in size of the living epithelium followed castration of the host rabbit, and a marked increase in the size of the implant followed treatment with massive doses of testosterone. Moore (1947) obtained similar results from the prostate gland of rats in situ.

Androgens from sources other than the testis have also been shown to influence the prostate. Thus grafts of ovary transplanted into the ears of castrated rodents will maintain the size and the normal histological appearance of the prostatic epithelium and seminal vesicles. Hill & Strong (1938) attributed the androgenic effect of the ovary to the lowered temperature of the ear, and Deanesly (1938) has correlated this activity with extensive luteinization of the theca interna within the ovarian grafts.

The classic experiments of Huggins and his associates (Huggins, Masina, Eichelberger & Wharton, 1939; Huggins & Clark, 1940) on prostatic secretion in dogs gave a clearer understanding of the functional behaviour of the prostate. They found that secretion in dogs is suppressed by oestrogen and maintained at a reduced level by gonadotrophin and oestrogen combined. It is restored to normal by testosterone in animals previously castrated or injected with oestrogen (Huggins, 1956). The conviction is now widely held that hormones which inhibit prostatic function will also cause remission of prostatic neoplasia.

In rare instances androgen-dependent prostatic cancer can be induced in rodents without the direct intervention of a hormonal agent. Thus a glandular-cell prostatic carcinoma has been obtained by Horning (1949) in the mouse, and by Mirand (1956) in the rat, by the administration of a carcinogenic hydrocarbon, 20-methylcholanthrene. The mouse tumours grew as grafts in castrated mice only if the host rodents had been treated with testosterone. During serial grafting, however, these hormone-dependent glandular neoplasms underwent squamous metaplasia. Once this change had occurred they ceased to be dependent upon androgen for their maintenance.

b. Effects of Oestrogen on the Prostate

As a general rule, cancer of the prostate develops spontaneously only in elderly men and old dogs. Apparently it does not arise in other mammals; but a condition similar to benign enlargement of the gland can be induced in most mammals by the application of massive doses of oestrogen. This effect has been studied extensively in rodents by Burrows & Kennaway (1934), Burrows (1949) and others. Burrows described in detail the progressive metaplasia induced by oestrogen and noted the sequence in which these changes occurred in different regions of the gland. With the possible exception of the golden hamster (Horning & Whittick, 1954; Horning, 1958), there is no evidence that exogenous oestrogens cause prostatic malignancy in rodents (Burrows & Horning, 1952, p. 43), although squamous metaplasia of the epithelium, accompanied by similar changes in the male accessory glands, may be induced. Horning & Whittick (1954) reported hyperplasia of the alveolar epithelium in the ventral prostate of oestrogen-treated hamsters. However, the metaplastic changes induced in rodents following oestrogen treatment are generally not identical with the lesions occurring spontaneously in man and the dog. Three other hamsters treated for prolonged periods with stilboestrol developed sarcoma from the fibromuscular stroma of the gland.

Zuckerman (1938) has shown that in dogs prostatic hypertrophy and metaplasia can be produced following inoculations of oestrogen, which can be prevented by testosterone administration. In dogs and in man where cancer of the prostate develops spontaneously during senility there is evidence that excessive amounts of oestrogen may be the cause of this disease (De Jongh, 1935; Burrows, 1935), but there is no evidence that endogenous oestrogens play a similar role in prostatic carcinogenesis of rats and mice. This is of interest in view of the fact that the rodent prostatic epithelium is capable of undergoing malignant changes in response to carcinogenic hydrocarbons.

c. Adrenocortical Hormones and Prostatic Cancer

There is experimental evidence to support the view that an endocrine dysfunction is involved in prostatic cancer, and that extragonadal hormones influence prostatic growth. Although adrenocortical tumours occur sporadically in most mammals, they are rare in untreated intact mice. Howard (1937, 1938)

showed that castration in young male rats stimulated the adrenal cortex to secrete androgens which, according to the experiments of Price (1941), are capable of maintaining the normal appearance of homologous intra-ocular grafts of prostatic epithelium.

In order to induce in mice tumours of the adrenal cortex which secrete sex hormones, two factors are essential—mice of a suitable inbred strain and the removal of the gonads at an early age. In the ce strain of mice of Woolley & Little (1946), both sexes developed cortical carcinomata within seven months of gonadectomy. The tumours produced either oestrogenic or androgenic responses in the host, and many of the tumours metastasized or were transplantable. That these cortical carcinomata secreted sex hormones was demonstrated by the changes in the mammary glands, uterus and clitoridean glands of ovariectomized females which were typical of oestrogenic stimulation. Castrated males with adrenal tumours had enlarged seminal vesicles and prostate glands. Gardner (1941) obtained similar results in other strains of mice under the same conditions and later (Gardner, Pfeiffer, Trentin & Wolstenholme, 1953) suggested that, in the absence of gonadal steroids, the pituitary-adrenal relationship is altered with the result that cortical carcinomata develop.

9. Tumours of the Uterus

Lipschütz (1950), who with his pupils has contributed so much to our understanding of tissue reactions to steroids, has reviewed the literature on experimental tumours of the uterus. Interest in the role of oestrogens in their pathogenesis continues (Williams, Paulus & Erikson, 1953; Gardner & Ferrigno, 1956) (see Plate I, fig. D). Meissner, Sommers & Sherman (1957) saw in rabbits treated with stilboestrol progressive changes not unlike those postulated to precede endometrial cancer in women (Hertig & Sommers, 1949), namely hyperplasia, adenomyosis and polyps. They obtained six endometrial carcinomata and a variety of benign lesions. Of special interest were the changes seen invariably in the endocrine glands (Sommers & Meissner, 1957), including enlarged pituitaries, adrenals and ovaries—evidence for a rather complex hormonal imbalance. Likewise, the endometrial carcinomata which developed, together with other neoplastic lesions of the genital organs, in guinea-pigs subjected to ovarian fragmentation (Bruzzone & Lipschütz, 1954) are the result of a long-continued ovarian-hypophysial imbalance.

The assumption that the various natural or synthetic oestrogens act in a more or less analogous manner on target organs now seems an over-simplification. The growth response of the uterus to oestriol differs from that to oestradiol (Dorfman, 1940), and oestriol inhibits the action of oestradiol and oestrone on the uterus (Hisaw, Velardo & Goolsly, 1954). Huggins & Jensen (1955) tested a series of steroids and found that several 3-hydroxyoestratriene derivatives behaved like oestriol. Long-term experiments with these "impeded" oestrogens would be of considerable interest.

Homburger, Borges & Tregier (1957) obtained sarcomata of the myometrium by administration of testosterone to young mice, the cervix of which had been ligated.

10. Cancer of the Breast

Experimentalists and clinicians agree that sex hormones of gonadal (see Plate II, fig. F) and adrenal origin play an important part in the development of tumours of the breast, but the role of pituitary factors is less well understood. Since lack of space prevents a comprehensive discussion of all hormonal factors, only the latter will be considered.

Pituitary Factors

Loeb & Kirtz (1939) were the first to demonstrate that pituitary grafts promoted the development of mammary carcinomata in virgins of strain A. Breeders of this strain have a high incidence of breast cancers but virgins a low incidence. Wolfe, Kirtz & Loeb (1940) obtained histological evidence that such grafts survive and consist of acidophilic and chromophobic elements. These results were confirmed and extended to mice lacking the milk factor (Mühlbock, 1956).

Evidence accumulated that anterior lobes grafted to sites remote from the hypothalamus secrete mainly prolactin and small amounts of ACTH (Everett, 1954, 1956; Mühlbock & Boot, 1956). Apart from oestrogen and progesterone, two hormones of the adenohypophysis are necessary for the full development of mammary glands of gonadectomized, hypophysectomized rats: growth hormone and prolactin (Lyons, Li & Johnson, 1952). Since only the presence of the latter has been demonstrated in pituitary grafts, one is tempted to attribute to prolactin the action of transplanted hypophyses which promotes mammary tumours. Bielschowsky *et al.* (1956) noted that breeders of the NZY strain showed a greater degree of hyperplasia of one type of acidophilic cells—the mammotrophs—and a higher incidence of mammary cancers (see Plate II, fig. E) than virgins, an observation pointing in the same direction, and Furth, Clifton, Gadsden & Buffett (1956) observed neoplastic changes in the breasts of rats bearing transplants of mammotrophic pituitary tumours.

11. Endocrine Therapy in Human Cancer

The carcinogenic action of certain hormones and the mechanisms which ultimately lead to cancer have been briefly discussed. Thus a specialized group of neoplasms exists whose growth can be influenced by endocrine therapy, and knowledge gained by animal experimentation has been of direct value in the application of therapeutic measures for the control of hormonal cancer in man. The work of Woolley on the induction of adrenal tumours in mice gave important information on the role played by steroid hormones in this problem. Huggins & Scott (1945) were the first to advocate gonadectomy combined with bilateral adrenalectomy for the treatment of breast and prostatic cancer. With the advent of cortisone, removal of the adrenals became a possibility and this is now a widely practised form of treatment. Remissions vary from several months to three years and in exceptional cases for longer. This procedure has been used extensively by Cade (1954) who found improvement in about 30% of a large series of cases of breast cancer treated in this way. In some patients the disappearance of bone metastases, followed by a complete recalcification, is often very dramatic (see Plate II, fig. H).

Recurrences of tumour growth after periods of remission following both gonadectomy and adrenalectomy suggest that the anterior pituitary may be capable of stimulating the adrenal rests to secrete oestrogens in sufficient amounts to reactivate the neoplasms, or it may be that the reoccurrence of some breast carcinomata is due to a pituitary hormone or hormones acting in addition to oestrogen. Hypophysectomy without previous gonadectomy and adrenalectomy was

introduced by Luft & Olivecrona (1953) for patients with breast cancer. This form of treatment is now used in many centres and has produced improvement in patients with cancer of the breast and in some cases with cancer of the prostate.

The fact that some forms of endocrine cancer can be fully or partly controlled by alterations of the hormonal environment is, to say the least, encouraging. It would be pertinent to conclude by quoting a recent remark by Dr Jacob Furth (1955): ". . . recognition and correction of the hormonal disturbance [in this disease] is, in fact, cancer prophylaxis."

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COCARCINOGENESIS

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1 Theories of tumour development based on the use of cocarcinogenesis
2 Advances since 1947
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The term cocarcinogenesis, introduced by Shear (1938) to describe enhancement by a basic fraction of creosote of the carcinogenic effect of 3:4-benzopyrene, has since been used to cover a wide range of phenomena in which tumours are produced by the action of more than one agent, acting either together or serially. Research and clinical observation in many parts of this field have added to our understanding of the genesis and development of tumours; but the most precise information has been gained from the study of cocarcinogenesis in skin, and it is to this study that the present survey of recent advances will be chiefly devoted.

1. Theories of Tumour Development Based on the Use of Cocarcinogenesis

When the subject was last reviewed in this Bulletin (Berenblum, 1947) eleven years ago, trends of thought and investigation had been formed which have continued till today. The work of Rous and his colleagues (Rous & Beard, 1935; Rous & Kidd, 1939, 1941; Friedewald & Rous, 1944a, 1944b) on rabbit skin, and of Mottram (1944a, 1944b, 1945), Berenblum (1941a, 1944), and Rusch & Kline (Kline & Rusch, 1944; Rusch & Kline, 1946) on mouse skin, had led to theories of carcinogenesis which were essentially similar. All were agreed that the process was divisible into stages. The initial stage consisted in the conversion of some normal tissue cells into what were variously described as, for example, " latent tumour cells ", or " sensitized cells ", and these cells, after a period of latency, began to multiply and form tumours during the second stage. A third stage, the development of malignancy, was postulated by Berenblum (1941b) and by Rous & Kidd (1941) but has not been generally accepted as such, owing to our ignorance of when this change occurs, or is determined, in relation to the other two stages.

After some divergence, the terminology of Rous has been adopted, namely " initiation " and " promotion " for the two stages, and " latent tumour cells " for the altered cells formed during the first stage.

The original reports on which these theories were based have been fully reviewed (see, for example, Berenblum, 1954b; Foulds, 1954). Rous and his colleagues relied mainly on observations on the effects of limited application of tar or carcinogenic hydrocarbons to rabbits' ears. Most of the tumours which arose disappeared when treatment was stopped, but reappeared, often at the same sites, when treatment was resumed either with the original agent or with non-carcinogenic irritants such as turpentine or chloroform. When penetrating wounds were made in an ear treated for a short time with a carcinogen, tumours arose in the scars. Rusch & Kline, Mottram, and Berenblum & Shubik, all observed the effects of non-carcinogenic irritants applied to the skin of mice before, with, or after, carcinogenic hydrocarbons. Berenblum and Shubik expressed their conclusions in a particularly precise way, and it will be convenient to take their version as a starting-point for the account which follows. They described a " model experiment " (Berenblum & Shubik, 1947) which has in fact provided a model for many subsequent experiments. A single dose of a carcinogen (the initiating stimulus) was applied to the skin of mice and followed, after varying intervals, by repeated application of croton oil (the promoting stimulus). Tumours appeared after an average latent period (beginning of promoting stimulus to appearance of first tumours) which did not change significantly with changes in the dose of carcinogen or in the length of the interval between the initiating and promoting stimuli. The proportion of tumour-bearing mice rose to a maximum, short of 100%; this " set level ", as they called it, was dependent on the concentration of the initiating dose, but independent of the length of the interval between initiating and promoting stimuli. Control experiments showed that the initiating and promoting stimuli used produced only an occasional tumour when applied separately, or in the reverse order. Berenblum and Shubik concluded that an initiating stimulus converted a relatively small number of normal cells into latent tumour cells, and that this change was irreversible. These cells, and these only, gave rise to tumours under the influence of the promoting stimulus. General changes in the treated skin (for example, the characteristic but transient hyperplasia following single applications of carcinogenic hydrocarbons (see Pullinger, 1940, 1941; Glücksmann, 1945)) were regarded as side-effects.

There has been much speculation about the cellular changes underlying the stages of carcinogenesis. Initiation, it has been generally agreed, must be a heritable cellular change, but objections have been made to calling it a mutation (Rous & Kidd, 1941; Berenblum & Shubik, 1949). Two related theories have been put forward to account for the special behaviour of initiated cells when acted on by promoting agents. According to Rusch (1954) the essential process in the stage of initiation is the suppression of an enzyme-forming mechanism. During subsequent cell-divisions (accelerated by promotion) this deficiency leads to further losses of such mechanisms, owing to a breach of metabolic chains. The final results are the loss of many if not all of the differentiated functions of the cell and the diversion of cellular nutrients to the reproductive mechanism, which then proceeds unchecked.

In Berenblum's theory (1954a) initiation is an irreversible change of a few of the " stem cells " of the tissue to latent tumour cells, which do not proceed at once to form a tumour only because they are isolated and surrounded by normal cells. Promoting agents have the two actions of increasing mitotic frequency and delaying maturation (whereas non-promoting

irritants have only the former). All the stem cells are induced to divide rapidly, and the maturation of their daughter cells is delayed so that more than the usual 50% of them divide again before differentiating. Thus the relative and absolute numbers of stem cells increase, as Glücksmann (1945) had shown by actual cell counts, and among these the latent tumour cells form compact clusters which reach a critical size and then proliferate without further stimulus.

In both these theories stimulation of mitosis and resulting hyperplasia are parts of the process of promotion (though, according to Berenblum, a minor part). It is conceivable, however, that the hyperplasia caused by known promoting agents is incidental. There seems no doubt that tumours occasionally arise in non-hyperplastic tissue, and remembering this we may hope to find promoting agents (as we have already found initiating agents—see section 2a below) which do not cause hyperplasia of the tissue as a whole.

2. Advances since 1947

Among the researches in recent years on aspects of cocarcinogenesis, two groups in particular have influenced current ideas of tumour development. Firstly, search for new initiating and promoting agents has been rewarded by the discovery of active substances of great theoretical and practical interest. Secondly, the methods and materials of the earlier investigators have been subjected to analysis both by themselves and by others, with the result that the clear lines of their first conceptions have been partly blurred, and a situation has been revealed which is more complex than was at first supposed.

a. New Initiating Agents

The initiators used in the experiments already described were all carcinogenic hydrocarbons, and demonstration of their initiating action required the use of subcarcinogenic doses. In 1953 Graffi and his colleagues in Germany (Graffi, Vlamynck, Hoffmann & Schulz, 1953) and Salaman and Roe (Salaman & Roe, 1953; Roe & Salaman, 1954) in London found that when urethane was applied to mouse skin, followed by, or alternating with, croton oil treatments, many benign and some malignant tumours appeared. This substance produces adenomata of the lung in mice when applied by any of a variety of routes, including the percutaneous (Nettleship & Henshaw, 1943; Cowen, 1950). After 18 weekly applications to the skin of stock mice no skin tumours were observed at five months, and no histological changes were found at any time in the treated skin (Roe & Salaman, 1954). However, Lindsay (1956) has recently reported from New Zealand that when male mice of the NZY inbred strain were given one or two skin applications of urethane, and then observed for 18 months without further treatment, 24 skin tumours, including one squamous carcinoma and six sebaceous adenomata, appeared on 11 of 15 treated mice. This experiment should be repeated in other strains of mice, because the NZY strain has peculiar genetic characters, including a tendency to obesity, an endocrine imbalance, and a fairly high spontaneous incidence of various types of tumour, some rarely seen in other strains (Bielschowsky, 1956; Bielschowsky & Bielschowsky, 1956).

Examination of a large number of other substances (selected for the most part because of carcinogenicity for other tissues, or pharmacological or chemical similarities with urethane) has resulted in the detection of several other initiators (Graffi *et al.* 1953; Salaman & Roe, 1953; Roe & Salaman, 1955; Salaman & Roe, 1956a; Roe & Glendenning, 1956), notably 1 : 2-benzanthracene, β-propiolactone, triethylene melamine (TEM), and 4-*NN*-di-(2-chloroethyl)-aminophenylbutyric acid (CB1348). Repeated application to mouse skin showed that of these only β-propiolactone was carcinogenic, and that TEM, like urethane, produced no histological changes. An incidental finding was that several non-initiating substances, notably nitrogen mustard and 2-*NN*-di-(2-chloroethyl)-naphthylamine (CB1048), produced a type of hyperplasia of mouse epidermis previously thought characteristic of carcinogenic hydrocarbons (Pullinger, 1940). A recent test of nine esters of methanesulphonic acid, including the anti-leukaemic agent Myleran (Roe, 1957), showed that four had initiating power, although Myleran was inactive. The most active of these, allyl methanesulphonic acid, produced no histological changes; some of the others (active and inactive) had effects like those of nitrogen mustard and the carcinogenic hydrocarbons. Thus, whatever may be the significance of this type of hyperplasia, it is not characteristic of initiation.

Acetamidofluorene is not an initiator for mouse skin when applied to it directly (Graffi *et al.* 1953; Salaman & Roe, 1953), but does have this action when given by mouth (Ritchie & Saffiotti, 1955). Moreover urethane, when given by mouth or parenterally, has an initiating effect on mouse skin (Berenblum & Haran, 1956; Berenblum & Haran-Ghera, 1957a). TEM and β-propiolactone have been shown to have a similar action (Roe & Salaman, 1956), as have several carcinogenic hydrocarbons (Berenblum & Haran-Ghera, 1957b). The fact that the ingestion, or parenteral injection, of these substances produces an undetectable effect on a distant tissue, which is revealed by the appearance of tumours only after the later application of a local stimulus, is clearly of great potential importance for the future study of " spontaneous " tumours.

Initiation by physical agents has been demonstrated. Burning (Saffiotti & Shubik, 1956), and large doses of β radiation (Shubik, Goldfarb, Ritchie & Lisco, 1953), followed by croton oil treatment of mouse skin, yield significant numbers of tumours. β Radiation alone produces a few malignant tumours after a long latent period in mouse and rat skin (Glücksmann, 1951[1]; Glücksmann & Boag, 1953). Since these physical treatments cause gross damage and extensive scarring, they are perhaps less suitable as tools for the investigation of the initiating process than some chemical agents.

b. New Promoting Agents

Several attempts have been made to isolate from croton oil the active principle responsible for its tumour-promoting action. Following studies by Cherbuliez and his colleagues (Cherbuliez, Ehringer & Bernhard, 1932), Berenblum found that about a fortyfold concentration could be effected by solvent partition (Berenblum, 1941a). With this as starting-point, Gwynn (1955) separated a number of fractions by chromatography, all of which, like the parent oil, produced gross epidermal hyperplasia, but only two had promoting power. The most active of these represented a concentration of from fiftyfold to a hundredfold. This is not a pure substance, and efforts to characterize the active principle suffi-

[1] See also Glücksmann, p. 178 of this number of the Bulletin.—Ed.

ciently closely to enable analogues to be tested have so far failed. Tests in the same laboratory of a large number of other substances for promoting power resulted in the detection of two, iodoacetic acid and chloroacetophenone, with moderate activity (Gwynn & Salaman, 1953; Gwynn, 1956). Both of these react with SH groups, but another compound of this class, iodoacetamide, proved inactive. All the active substances were irritant and hyperplasia-producing, but, as was expected from previous work (Berenblum, 1944; Shubik, 1950a), many other equally irritant and hyperplasia-producing substances were not tumour-promoting.

High concentrations of phenol and of several of its analogues have been shown to be promoting agents for mouse skin by Rusch and his colleagues (Rusch, Bosch & Boutwell, 1955; Boutwell, Bosch & Rusch, 1957). This has been confirmed by Salaman & Glendenning (1957) who found also that intradermal injections of sclerosing agents (proflavine, ethanolamine oleate, phenol) were tumour-promoting. These procedures all produce damage and hyperplasia in both epidermis (with its appendages) and dermis, and this raises the important question what role is played by the dermis in epidermal carcinogenesis. On this matter, some aspects of which are discussed by Professor Orr on p. 99 of this symposium, decisive evidence is, I think, still lacking.

Several fractions of catalytically cracked mineral oils (Shubik & Saffiotti, 1955) and a straight-chain hydrocarbon, *n*-dodecane (Shubik, Saffiotti, Feldman & Ritchie, 1956), have been shown to be tumour-promoting, and it seems likely that other substances of this class may be active. Setälä and his colleagues (Setälä, Setälä & Holsti, 1954; Setälä, 1956) have shown that several lipophilic-hydrophilic surface-active agents are tumour-promoting, notably polyoxyethylene sorbitan mono-palmitate, -stearate, and -oleate (Tween 40, 60, and 80) and sorbitan monolaurate (Span 20). These important discoveries provide new classes of tumour-promoting substances with well-defined physical properties, and may lead to rapid advances in our understanding of the promoting process. But it should be noted that the potency of the active principle of croton oil is of a much higher order, and further purification and analysis of croton oil fractions is therefore much to be desired.

c. Practical Limits to the Separation of Initiating and Promoting Actions

One of the assumptions made by Berenblum and Shubik was that the initiating and promoting treatments which they used were incapable of producing tumours when applied alone. Their control tests were in fact negative, but it appears that this was an exceptional result. Several investigators here and in the United States of America have observed that single small doses of carcinogenic hydrocarbons such as Berenblum and Shubik used may produce a few benign and malignant tumours in mouse skin after long latent periods, and that repeated applications of croton oil, in even lower doses than theirs, may do so too. The magnitude and time relations of these effects have been described in recent reports (Roe, 1956a, 1956b; Salaman & Roe, 1956b), where former work is reviewed. It is not difficult to allow for a small number of tumours due to the separate action of initiators or promoters in tests for new agents of either of these classes, so long as experiments are properly designed and suitable controls included (see Roe & Salaman, 1955; Berenblum & Haran-Ghera, 1957a, 1957b). In urethane, TEM, and perhaps some others, we have initiators which appear to be almost non-carcinogenic for skin. It may be that some of the recently described promoting agents are quite non-carcinogenic (i.e., have no initiating action as well) but this has still to be proved. Most known initiators and promoters have some degree of the other action as well, but it is beyond question that the effect of their successive application is very much more than additive.

3. Present Position

a. Status of the Theory of Stages in Carcinogenesis

It has been said that before the formulation of this theory carcinogenesis was regarded as a single long-drawn-out process (Berenblum, 1954a). Whether or not such a view was ever general, it is certain that many familiar clinical and experimental data are well fitted by a theory of stages. Tumours which begin to grow suddenly after a long and uneventful latent period are at least as common as those which arise as the culmination of what seems a gradual process; and it is always possible that the latter conceals the essential sudden steps among more obvious but irrelevant changes. Besides, if it may sometimes be difficult to observe distinct stages in carcinogenesis before tumours arise, the "progression" of the tumours themselves, to use the term employed by Foulds (1954), is frequently obvious. Sudden increases in growth-rate, invasiveness, and independence of hormonal control, have been observed in a wide variety of tumours (Foulds, 1949, 1950; Shubik, Baserga & Ritchie, 1953). In human tumours the sudden acquisition of resistance to the action of a previously effective therapeutic agent is only too familiar.

Thus, though many difficulties remain, the theory of stages in carcinogenesis continues to receive support. Overt discontinuities in a process do not of course exclude the possibility of an underlying continuous change. The observed stages may simply manifest the attainment at the subcellular, cellular, or tissue level of threshold states at which successive lines of defence are breached. That the discontinuities can in some cases be induced by specific stimuli may mean that these stimuli enormously accelerate a continuous though fluctuating process. These considerations do not lessen the importance of the observed facts, but they show the possibility of alternative explanations and may suggest ways of distinguishing between them.

Whereabouts in the carcinogenic process is the malignant change determined? Berenblum (1941b, 1944) and Rous & Kidd (1941) regarded it as a third stage, following that of promotion. Shubik, Baserga & Ritchie (1953) have suggested that the character of potential malignant change is determined, together with a variety of other characters, at initiation, to be manifested later, during promotion. Foulds (1949) regards the development of malignancy as one of several independent progressive changes which occur during the life of a tumour.

A feature of the type of skin carcinogenesis which we have been considering suggests a promising method of attacking this difficult problem. Almost all the tumours produced by a single application of an initiator followed by repeated applications of croton oil to skin are benign warts, whereas many malignant tumours as well as warts are rapidly produced when a carcinogen is applied repeatedly. This difference has been examined and discussed by Shubik (1950b) and by Salaman & Roe (1956b). If mice treated by the former method

are watched for a long time, malignant tumours accumulate slowly, but at any given time they are fewer than in mice treated repeatedly with the carcinogen, though more numerous than in those which received the initiating dose alone. Observation is limited by death, and comparison with the much more rapid production of malignant tumours by the latter method is difficult. Further investigation of this difference is urgently needed. Comparison of the effects of single and multiple initiating stimuli, of successive applications of different initiators, and of their combination with different promoters, may well throw light on the place of the malignant change in the carcinogenic process.

b. Status of the Latent Tumour Cell Theory

The most influential concept of those we have considered is that of the irreversible transformation, at the initiating stage of carcinogenesis, of a definite number of normal cells into latent tumour cells. The evidence upon which Rous and his colleagues mainly relied was qualitative, namely the fact that tar-induced tumours of rabbits' ears which had regressed were made to reappear at the same sites by a variety of stimuli, not necessarily carcinogenic. Berenblum and Shubik, however, claimed that their observations (summarized in section 1) provided quantitative proof of the fact that a given initiating dose converts irreversibly a certain number of cells to latent tumour cells: when all these have been made to grow into tumours by the promoting treatment, no more tumours appear; hence the " set level " of incidence and its dependence on initiating dose.

Subsequent observations have given rise to modifications of the theory. Further, it appears that the quantitative evidence adduced for it can be interpreted in other ways. Friedewald & Rous (1950) modified the concept to account for the gradual accumulation of tumours on the ears of rabbits for as long as five years after limited treatment with methylcholanthrene. They showed that this increase was the resultant of a steady appearance of new tumours and a steady disappearance (at a slower rate) of older ones, and concluded that the stage of initiation was better described as the creation of " latent neoplastic potentialities " in the skin rather than of latent tumour cells. Shubik, Baserga & Ritchie (1953) introduced another modification, to explain the variety of characteristics seen in tumours produced by initiating and promoting treatment of mouse skin. While retaining the " latent tumour cell " postulate, they suggested that these cells were not all similar, but differed over a range of growth potentials, thus giving rise on promotion to tumours of different properties, for example, histological structure, growth-rate, and invasiveness. Similar suggestions have been made by Klein (1956) and by Vesselinovitch & Gilman (1957). In another experiment Shubik & Ritchie (1953) showed that the relation of the " set level " of tumour incidence to dose of initiator, which had been demonstrated for single applications, did not hold when the initiator was applied repeatedly. Both two and three applications of the initiator gave actually a slightly lower tumour incidence than a single application.

In experiments with various initiators and promoters in this laboratory a set level of incidence has not often been attained. Incidence (whether measured as percentage of tumour-bearers, or more informatively as the number of tumours per surviving mouse) has sometimes risen continuously, sometimes reached a peak and then fallen, and sometimes reached and maintained a more or less constant level, during continued promoting treatment. The reason for this seems to be that in the mouse, as in the rabbit (Friedewald & Rous, 1950), along with the continued appearance of new skin tumours there is a constant regression and disappearance of old ones (Roe, 1956a; Shubik, 1950b; Klein, 1953, 1956). The relative preponderance of these processes varies, hence the varying result: increase, decrease, or approximate equilibrium. Any cause of illness and debility, including old age, accelerates the regression rate; and it is when the mice are getting old that the set level is being looked for.

Further, it was implicit in Berenblum and Shubik's formulation that the attainment of a set level of tumour incidence meant that promoting treatment had been adequate, i.e., sufficient to cause every latent tumour cell to form a tumour. However, even on those rather rare occasions when approximately constant maximum incidences have been observed, as in some experiments of Gwynn & Salaman (1951) in which the same dose of an initiator was followed by treatment with different samples of croton oil and croton oil fractions, the levels attained varied. Thus the height of the set level depended not only on dose of initiator, but also on potency of promoter.

Somewhere in this complex situation proof of the existence of latent tumour cells may perhaps lie hidden, but it is hard to discern. We shall be wise to regard the theory as still unproven. Rous's conception of " latent neoplastic potentialities " includes it, and is both wider and more adaptable, but still appears to contain the hypothesis of an all-or-nothing change limited to individual cells. Shubik's suggestion of a limited number of initiated cells with graded growth potentials, an important modification, contains the same unproven postulate.

All that the facts permit us to say is that an initiating stimulus produces a permanent alteration in the state of the skin, such that there is a heightened probability that tumours will arise in it under certain conditions, for example as the result of promoting treatment, or occasionally spontaneously under the influence of normal environmental factors. We may add the supposition that this probability is higher in some places than in others—that it varies over a range of levels. To make the hypothesis more specific than this seems unnecessary. The peaks of probability may be quite steep, so that small groups of cells may be much more readily promoted than others, while regions of lower and lower probability will require progressively more potent or prolonged promoting treatment. Differences in persistence, growth-rate, and power of progression, in the resulting tumours may also depend on different degrees of initiation, not necessarily linked to those responsible for degrees of promotability. Such a theory includes that of the latent tumour cell as a special case, for the probability peaks may sometimes be so sharp that the power to respond to a particular promoting treatment is confined to a few isolated cells.

Whether such a sharply localized effect is common is a question too wide to be adequately dealt with here; it involves the old controversy of regional versus focal origin of tumours, which is far from decided. Certainly focal origin is by no means obvious histologically in mouse skin. Small tumours often show a structure very difficult to interpret as the result of proliferation of a single cell, or even of a small group of cells. A region of hypertrophied and altered epidermis, hair follicles, and dermis, is seen, all apparently taking part in the

same process. In other cases focal origin sometimes seems likely or at least possible; sometimes regional origin is much more probable. Like many other theories of carcinogenesis originally proposed to cover a certain range of phenomena, the " latent tumour cell " theory is not necessarily valid for other ranges. It has proved a very stimulating concept, but it should not be allowed to harden into a dogma while unequivocal evidence in its favour is lacking, and while there are types of carcinogenesis to which it is inapplicable in its present form.

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AVIAN CARCINOGENESIS

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Although tumours of many histological types similar to those of mammals have been described in many species of birds, only those of the domestic fowl have been extensively investigated. It follows therefore that most of our knowledge of carcinogenesis in birds is based on experiments on chickens, though ducks, geese, pheasants, turkeys and guinea-fowl have been used on a smaller scale.

1. Infective Agents

The first successful induction of sarcoma under experimental conditions was the well-known transmission by cell-free extract of a fowl sarcoma of spontaneous origin by Rous (1911). Similar results were obtained by Rous and his associates with several other spontaneous sarcomata of fowls, and subsequent work in many parts of the world confirms the presence in some spontaneous fowl tumours and leucoses of specific causal agents having many of the properties of viruses.

Reviewing the evidence, Claude & Murphy (1933) concluded that all the spontaneous sarcomata of fowls that had been successfully propagated by cell grafts had eventually been shown to carry specific infective causal agents. Murphy preferred, however, to call these agents " transmissible mutagens " in order to avoid classing them as viruses. Whatever the fundamental nature of the " infective agents ", as Rous called them, there is good evidence that they are particulate, thermolabile and antigenic. They initiate in a new susceptible host a histologically specific tumour from which increased amounts of infective agent can be recovered.

A feature common to several such infective sarcomata of different origin is the presence of a mucoid viscous exudate which is so characteristic as to allow of immediate diagnosis of this type of tumour. The Mill Hill 2 endothelioma described by Begg (1927), though presenting a completely different histological and macroscopic appearance, is also transmissible by cell-free extracts.

Whether the original tumours from which these specific infective agents were extracted were caused by infection as generally understood, or whether the agents represent some self-reproducing cell organelle essentially intrinsic in origin has never been established.

2. Spontaneous Tumours

An interesting clue to aetiology was reported by McGowan (1937) who observed three cases of sarcoma at the site of weasel bites on the wings of chickens. McGowan commented on the " mucilaginous, ' tacky ' appearance " of the cut surface of two of these tumours. It is unfortunate that he seems not to have investigated them for infective agents. As most weasel bites are fatal it is probable that the localization of sarcoma in the wing is fortuitous and due to the failure of the weasel to retain its grip on the bird. McGowan, in a scholarly discussion of the aetiology of sarcoma, drew attention to traumatic and inflammatory reactions as a necessary precursor of malignancy.

I encountered one case of spontaneous fibrosarcoma of the back of a Barred Plymouth Rock fowl at a site of trauma of unknown origin, which might have been similar to McGowan's cases as weasels had been observed near the chicken runs.

Specific infective agents have not been demonstrated in mammalian sarcomata, though the infective fibroma of the rabbit described by Shope (1932) is caused by a virus, and such tumours may become sarcomatous under experimental treatment with carcinogens as shown by Andrewes & Ahlström (1938). However no infective sarcoma has been obtained from such experiments, and the fowl sarcomata seem to occupy a unique position. Such a difference might be related to the essential differences between birds and mammals, and some support for such a concept may be found in the Fujinami sarcoma which is transmissible to ducks and in the variants of the Rous Sarcoma No. 1 developed by Duran-Reynals (1942) which will also infect ducklings.

More recently Harris (1954) has successfully infected turkey poults with Rous sarcoma virus and has shown that the agent becomes antigenically more " turkey " than " fowl " with progressive passage in turkeys. Moreover, both erythroblastosis and myeloblastosis in fowls are transmissible by cell-free infective agents. Such considerations might suggest that all fowl tumours contain specific infective agents, but experience does not support this concept.

Another possibility is that most avian tumours are not caused by cell-free infective agents but by chemical or physical carcinogens capable of initiating a neoplastic change in a previously normal cell. Similar neoplastic changes might be caused by viruses which would then by definition be able to cause the same tumour in a new host.

The successful transmission of spontaneous sarcomata would be attributable to a specific virus, while those that were not transmissible even by cell graft might contain no virus. This concept of multiple causation of histologically similar tumours is supported by the great majority of experimental results. Thus most attempts to transmit spontaneous tumours of fowls have failed. It is a surprising fact that carcinoma of the ovary, a relatively common spontaneous tumour in the fowl, has not been successfully transmitted. Indeed very few epithelial tumours of the fowl have been transplantable and the author has failed repeatedly to transmit such tumours even in closely related line-bred White Leghorns, in which tumour strains derived from chemically induced sarcomata grow in almost every case.

3. Chemically Induced Tumours

Murphy & Landsteiner (1925) induced sarcoma in two fowls by inoculation of embryo pulp and coal-tar. One of the resulting tumours was transplantable and was maintained

through 11 serial passages, but attempts to demonstrate a filter-passing infective agent failed. Subsequent work has abundantly supported the concept that such chemically induced tumours do not contain an infective agent.

When pure chemical carcinogens became available we began a series of experiments designed to test the hypothesis that all fowl sarcomata contain a filter-passing infective agent. Barred Plymouth Rock, Rhode Island Red, Brown Leghorn and White Leghorn fowls in turn were injected intramuscularly with carcinogenic polycyclic hydrocarbons, and in each breed sarcomata were readily induced at the site of injection (Peacock, 1933, 1935).

As the tumours occurred attempts were made to transmit them by cell grafts and by cell-free extracts prepared in many ways. Briefly, it may be said that only 17 of 63 sarcomata were successfully transmitted and only when intact tumour cells were inoculated. These tumours were called GRCH 1, 2, etc., after the Glasgow Royal Cancer Hospital. Cell-free preparations gave uniformly negative results. These experiments have been described in detail elsewhere (Peacock, 1935, 1946).

In a very similar series of 62 chemically induced sarcomata, Murphy & Sturm (1941b) transplanted 12 strains in serial passage but failed to find any evidence for the presence of cell-free infective agents.

It may be significant that leucosis was rarely encountered in the GRCH series and that, when it was present, transmission experiments were avoided.

4. Avian Leucosis Complex

Three forms of leucosis occur with varying frequency spontaneously in poultry flocks. Lymphomatosis is not certainly transmissible by cell-free agents, but erythroblastosis and myeloblastosis are readily transmissible and contain specific infective agents. These agents can also cause sarcoma when injected intramuscularly in a minority of fowls. Thus it seems likely that, in flocks with a high incidence of leucosis, inoculation from fowl to fowl might yield a small proportion of sarcoma cases. However, if viruses occasionally induce spontaneous sarcoma it would not be surprising if chemically induced sarcoma should occasionally contain an infective agent. In such a case the propagation of the tumour by cell-free extract would be as understandable as in the case of the spontaneous sarcoma.

Few claims to have propagated chemically induced sarcoma by cell-free extracts have been made and in some of them the claim does not bear critical analysis, but one of the tumours of McIntosh & Selbie (1939) seems to be a case of the kind postulated above. In this case the bird could not have been infected at the time of injection of the tar and must either have carried the virus before the injection or acquired it subsequently.

As Burmester & Gentry (1954) have shown that many poultry flocks carry visceral lymphomatosis, it seems probable that the virus was present before the carcinogen was injected.

5. Epithelial Tumours

Despite the ease with which sarcoma can be induced in fowls by parenteral injection of tar or of carcinogenic polycyclic hydrocarbons, almost all attempts to induce skin tumours have failed. We have reported such negative results and put forward the suggestion that structural differences between birds and mammals, for example the absence of sebaceous follicles in the former, might account for this lack of susceptibility to cutaneous carcinogenesis. Nevertheless, spontaneous squamous carcinoma of the skin has been reported, though usually affecting the legs and feet. As fowls, in common with other birds, have preening glands histologically similar to sebaceous glands, we attempted to induce tumours in these organs by implanting into their lumen pellets of several recognized cutaneous carcinogens, but with uniformly negative results (Peacock & Peacock, 1949).

6. Acetamidofluorene

The versatility of action of 2-acetamidofluorene (AAF) in mammals suggested that this carcinogen might be as effective in the fowl as in mammals. This proved to be the case, and a variety of epithelial tumours followed injection into the lumen of the crop of AAF in arachis oil or in aqueous suspension.

7. Methylcholanthrene

Injection of 0.5% solution of methylcholanthrene in arachis oil into the wall of the crop gave rise to an anaplastic carcinoma at the site of injection in one bird; in another a primary adenocarcinoma of the proventriculus; and in a third a hepatoma and renal adenoma. Attempts to transplant these tumours failed. In contrast with the short induction time for sarcoma following intramuscular injection of methylcholanthrene, 8–18 months, the induction time for the epithelial tumours was between 3 and 5 years (Peacock & Peacock, 1956). This is similar to the incidence of these types of tumour in man. Possibly the relatively high incidence of sarcoma in fowls generally reported may be due to the fact that few fowls are allowed to live as long as 3 years.

8. The Role of Solvent

When AAF dissolved in arachis oil was injected, the effect was localized and squamous carcinoma of the crop occurred in three birds (Peacock & Peacock, 1954). AAF similarly injected in aqueous suspension induced adenocarcinoma in the bronchus and kidney, hepatoma and squamous carcinoma of the palate and papilloma of the oesophagus (Peacock & Peacock, 1954). This is in keeping with earlier experience, both with mice (Peacock & Beck, 1938) and with fowls (Peacock, 1936; Murphy & Sturm, 1941a).

The mode of action of solvents has been the subject of much discussion (Dickens, 1947; Peacock, Beck & Anderson, 1949) and is not completely understood but, in the case of polycyclic hydrocarbons such as benzopyrene, retention of carcinogen at the site of injection with slow partition between solvent and surrounding cells favours local carcinogenesis, while rapid absorption of solvent and carcinogen is unfavourable to local carcinogenesis though it may lead to carcinogenesis in a target organ or tissue. Such remote action follows parenteral injection of AAF and of the azo dye 4-dimethylaminoazobenzene (butter yellow), to quote only two examples.

9. Hormonal Factors

Little is known about the causes of spontaneous carcinoma in birds, but recently Greenwood (1955) has reported a very high incidence of ovarian carcinoma in fowls maintained in a constant environment throughout their lives. Such birds lay continuously for 10½ months in the year and do not moult. Control sisters subject to the normal seasonal variations in climate have a period of rest from egg-laying during the three

months' moulting season, and these birds had no ovarian tumours. In this case no extrinsic agents appear to be involved but the abnormal hormonal conditions seem to be responsible for the tumours.

Hormonal influence on carcinogenesis is also evident in the experiments of Michalowsky (1928, 1929), who induced teratoma in the testicles of cocks by injections of zinc salts. These tumours arose some months after the injection but only when the experiment was begun in early spring. Bagg (1936) repeated Michalowsky's experiment with similar results but was able to induce tumours also during the summer months by injecting gonadotrophic hormone as well.

Anissimova (1939), a pupil of Michalowsky, transplanted some of these teratomata but the grafts were subject to seasonal factors which inhibited growth in the late summer. This type of seasonal influence had been noted by the author in grafting chemically induced sarcomata (Peacock, 1935) and by Murphy & Sturm (1941a), and is presumably related to the moulting period, when rapid involution of the ovary and oviduct normally occurs.

10. Naturally Occurring Carcinogens

The demonstration of Campbell (1956) of carcinogenesis in the liver of fowls fed with *Senecio jacobaea* followed similar results in rats described by Schoental, Head & Peacock (1954). As this plant, the common ragwort, is widespread throughout the temperate zone, it may be a natural source of extrinsic carcinogen, though in my experience fowls have shown no tendency to peck at ragwort growing in their runs.

11. Discussion

Undoubtedly the best-known fowl tumour is the Chicken Tumour No. 1 of Rous, generally known as the Rous sarcoma. Because of its ease of propagation and of its theoretical implications it has given rise to a vast amount of experimental work. Nevertheless it is a rare type of tumour in nature and should not be regarded as typical of all spontaneous fowl sarcomata. The great majority of the reported tumours in birds have never been investigated beyond a histological report. Duran-Reynals (1946a) described 14 sarcomata among 50 neoplasms of spontaneous origin in fowls from the same farm. Of four sarcomata in birds from 12 to 18 months old, only one was transplantable indefinitely and contained a causal virus. Of five sarcomata in chickens 2–18 weeks old, none could be transplanted indefinitely, though one was transplanted through two passages and two through one passage. Of five sarcomata in chickens from 5 to 10 months old, four were indefinitely transplantable and contained causal virus. In another seven sarcomata of equal or greater malignancy, no virus could be demonstrated. Duran-Reynals (1946b) also attempted to transmit 12 lymphoid tumours and one adenocarcinoma of the ovary without success.

My own experience is similar and suggests that most spontaneous fowl tumours resemble those of mammals in all their demonstrable characteristics. There is no evidence that most of these tumours are caused by viruses. On the other hand the spontaneous tumours that are readily transplantable usually contain specific viruses. However, transplantability and virus content are not necessarily concomitant, as shown in the case of the GRCH series and in chemically induced sarcomata described by Murphy & Sturm (1941b).

The question arises whether chemically induced sarcomata are comparable with spontaneous sarcomata, and there seems to be no way of assessing the relative merits of virus-induced or chemically induced tumours as models of spontaneous neoplasia. It must be admitted that many chemical carcinogens do not occur in nature, whereas all the virus-induced tumours are derived from spontaneous tumours. There are other reasons, however, for doubting whether the virus-induced sarcomata are typical of tumours in general.

Four separate virus-induced tumours, Rous No. 1, Fujinami, McIntosh 5 and Mill Hill 2, occasionally regress spontaneously. In the case of the Fujinami sarcoma, the same source of infective virus causes histologically identical tumours in fowls and ducks, but in the former most of the tumours grow progressively while in the latter many of them regress. Spontaneous cure is certainly not a common feature of sarcoma in other species.

In the birds bearing virus-induced tumours between 2 and 9 days after inoculation it is possible to localize similar tumours anywhere in the bird at the site of a small cautery puncture (Peacock & Peacock, 1953). No such localization occurs in birds bearing chemically induced sarcomata GRCH 16, 17 or 22.

Other reasons for considering the virus-induced fowl tumours to be essentially different from chemically induced tumours are the survival of infectivity in virus tumours after exposure to *x* rays or ultra-violet rays in doses which destroy the transmissibility of living cells, and the absence of evidence of cross-immunity between virus-induced and chemically induced sarcomata (Peacock & Peacock, 1953).

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SOME POSSIBLE EXOGENOUS FACTORS IN THE CAUSATION OF LUNG CANCER

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In the present state of knowledge we thought that the most useful contribution which could be made to this symposium would be, not an epitome of the whole mass of literature of which some items are familiar already, but a collection of data, as complete as possible, which would assist in the identification of the actual carcinogens.

The range of known chemical carcinogens is now so wide, and the new data about cocarcinogenic action open up such attractive considerations, that we decided to give lists as complete as possible of the compounds identified in the various forms of tobacco, and of tobacco smoke, and in the atmosphere. Naturally special attention is paid to the work, in part not yet published, carried out in London at the Sir John Cass College and at the Department of Pathology, St Bartholomew's Hospital. The position of atmospheric pollution in relation to cancer of the lung is difficult and uncertain, and it seems best to point out possibilities, of which some are not yet generally recognized.

1. Possible Carcinogens

Of the many extrinsic carcinogenic agents now known, those that may be responsible for pulmonary cancer may be either (i) radiations, or (ii) solid, liquid or gaseous substances. There is a high incidence of cancer of the lung in some occupations involving exposure to nickel, chromium, arsenic, beryllium and asbestos, but the contribution from these sources to the total number of deaths is small and they will not be considered in detail. All the data available at the time about cancer of the lung at Schneeberg and Jachymov in the Erzgebirge were given in an earlier paper (Kennaway & Kennaway, 1944). Apparently nothing further is to be learned now, because both mines are under direct or indirect Russian control, and the old long terms of service by local people have been replaced by much shorter periods of imported labour.

a. Radiations

The effects of radiations were summarized in a recent report to the Medical Research Council (1956). There is no evidence that external radiation by x rays or γ rays has caused lung cancer in man. The miners in the Erzgebirge inhaled air containing high concentrations of radon and also dust containing other radioactive elements; after a mean exposure of 17 years, during which time the dose reaching the lungs could have been equivalent to 1,000 r., about one-half of the miners died from lung cancer. The composition of this air showed that during the average period of 17 years the local dosage could have been as great as 10,000 r. if non-uniform distribution of the active particles occurred in the air passages.

b. Chemical Compounds

Large numbers of compounds of many types have been shown to be present in tobacco smoke and town air, and there are many more compounds as yet unidentified in both. Although these materials are so different, many partial combustion products found in each are identical and some of these are known to be carcinogenic.

2. Composition of Tobacco

If a causal factor is introduced by smoking, this may be a constituent of the tobacco itself or a substance produced during the smoking process. Much is known about the composition of tobacco and is recorded in standard technological works. The composition is variable and depends upon the variety of tobacco plant, the locality and soil conditions of growth, the mode and time of harvesting and the various processes of curing and ageing employed. A list of the constituents reported as present in tobacco is given (Table I); a subsidiary list of classes of compound that have been recognized, without identification of the individual compounds, is also given.

Table II gives the general composition of a typical flue-cured Virginia-type tobacco.

The polycyclic aromatic hydrocarbons anthracene, pyrene, fluoranthene and 3 : 4-benzopyrene were first shown to be present in tobacco in minute amounts by Campbell & Lindsey (1956), and it was concluded that atmospheric pollution might in part account for the presence of these compounds but that larger amounts found in a few types of tobacco originate from fire-curing, which involves hanging the leaves in closed barns where they are exposed to wood smoke for a long period.

3. Composition of Tobacco Smoke

The smoking of tobacco involves a number of fundamental processes which all give rise to products found in the smoke. These are: (i) combustion, producing oxides of carbon and water, (ii) thermal decomposition, occurring in or near the hot zone and giving rise to a number of products which are either subsequently burnt or distilled away into the mainstream smoke, (iii) distillation, proceeding in close proximity to the smouldering zone and giving vapours that condense in cooler zones, and (iv) steam distillation, occurring mainly

TABLE I. THE CONSTITUENTS OF TOBACCO

Individual elements and compounds

Acetaldehyde	Fructose	Nicoteine
Acetic acid	Fumaric acid	*iso*Nicoteine
Adenine	Furfural	Nicotelline
Alanine	Furfuryl alcohol	Nicotimine
Aluminium	Galacturonic acid	Nicotine
Ammonia	Gentiobiose	Nicotyrine
Anabasine	Glucose	Nornicotine
Anatabine	Glutamine	Nornicotyrine
Anthracene	Glycerol	Oleic acid
Arginine	Glycine	Oxalic acid
Arsenic	Guanidine	Palmitic acid
Ascorbic acid	Histidine	Phenylalanine
Asparagine	Hydrocarbons	Phosphorus
Barium	$C_{10}H_{16}$	Polyuronic acid (pectin)
3 : 4-Benzopyrene	$C_{10}H_{18}$	Potassium
Betaine	$C_{11}H_{20}$	Pyrene
Boron	$C_{27}H_{56}$ (Heptocosane)	Pyridine
*iso*Butyric acid	$C_{31}H_{64}$ (Hentriacontane)	Pyrogallol
Caffeic acid	Imidazole	Quinic acid
Calcium	Inositol	Rhamnose
Caprylic acid	Iron	Rutin
Carbon dioxide	Lactic acid	Silicon
Carotene	Leucine	Sodium
Catechol	Lignin	Solanesol
Cellulose	Linoleic acid	Sorbitol
Chlorine	Lithium	Starch
Chlorogenic acid	Lysine	Strontium
Chlorophyll	Magnesium	Succinic acid
Choline	Malic acid	Sucrose
Chromium	Maltose	Sulphur
Citric acid	Manganese	Tabacenic acid (α, β, γ)
Copper	Methanol	Terephthalic acid
Cystine	*N*-Methylanabasine	Titanium
Cytochrome	*N*-Methylanatabine	Tryptophan
Dextrin	*N*-Methylmyosmine	*iso*Valeric acid
Edestine	Myosmine	Xanthophyll
Eugenol	Myristic acid	Zinc
*iso*Eugenol		
Fluoranthene		
Formic acid		

Groups of compounds

Acids	Monosaccharides	Polysaccharides
Alcohols	Nitrates	Porphyrins
Aldehydes	Nucleic acids	Proteins
Alkaloids	Paraffins	Quinones
Amides	Pentosans	Resins
Amino acids	Phenolic acids	Resin esters
Amino compounds	Polyhydric alcohols	Tannins
Esters	Polyphenols	Terpene hydrocarbons
Humic acids		
Hydroxy acids		

during the suction period and responsible for carrying quantities of tobacco constituents and thermal decomposition products into the smoke at a relatively low temperature.

It therefore follows that many of the more volatile constituents of the original tobacco are found in the smoke and that, in addition, products of combustion and thermal decomposition are present. With the latter must be included subsequent reaction products which originate from primary decompositions.

Table III, subdivided in the same way as Table I, gives a list of compounds reported as present in tobacco smoke. A separate list of carcinogenic compounds found in tobacco smoke is given in Table IV.

TABLE II. COMPOSITION OF FLUE-CURED VIRGINIA CIGARETTE TOBACCO

Name of substance	Quantity (%)	Name of substance	Quantity (%)
Ash (see (ii) below) –	13.2	*(continued)* Nitrogen compounds (see (i) below)	11.1
Cellulose – –	9.0	Oxalic acid – –	1.0
Citric acid – –	0.6	Pectins – – –	10.7
Fructose – –	7.8	Polyphenols – –	2.0
Glucose – – –	11.0	Resins (total) – –	9.8
Hemicelluloses – –	1.6	Starch – – –	4.0
Lignin – – –	3.5	Sucrose – – –	4.2
Malic acid – –	10.1	Total –	99.6

i. Composition of total nitrogen compounds

Amino nitrogen –	0.2	*(continued)* Nornicotine – –	0.3
Ammonia and amides	0.7	Protein – –	7.1
Nicotine – – –	1.7	Unidentified – –	0.6
Nitrate – – –	0.5	Total –	11.1

ii. Composition of ash

Oxides and carbonates of metals:

Aluminium	Molybdenum*
Arsenic*	Nickel*
Barium*	Potassium
Boron*	Rubidium*
Calcium	Selenium*
Chromium*	Silver*
Cobalt*	Sodium
Copper*	Strontium*
Iron	Thallium*
Lead*	Tin*
Lithium*	Titanium*
Magnesium	Vanadium*
Manganese*	Zinc*
Mercury*	

Other components:

Chloride	Silicates
Phosphate	Sulphates

* Present as traces

Recent investigations at the Sir John Cass College and at the Department of Pathology, St Bartholomew's Hospital, have been concerned chiefly with (i) the phenols (Commins & Lindsey, 1956b) and (ii) the polycyclic aromatic hydrocarbons, present in mainstream tobacco smoke. (Mainstream smoke is the smoke drawn through the pipe, cigar or cigarette by the smoker.)

i. *Phenols* are well-known constituents of smoke, and a number were recognized in tobacco smoke before the new method of testing came into use (Commins & Lindsey, 1956a); this involves methylation of the mixed phenols and separation of the methyl ethers by alumina chromatography. The individual ethers detected in the successive fractions by ultraviolet spectrophotometry are those of phenol, *o*-cresol, *m*-cresol, *p*-cresol[1], 1-naphthol[1], 2-naphthol[1]; the three isomeric

[1] Compounds found in cigarette smoke for the first time during these investigations.

TABLE III. THE CONSTITUENTS OF TOBACCO SMOKE

Individual elements and compounds

Acenaphthene	1 : 2-7 : 8-Dibenzonaphthacene	Methyl ethyl glyoxal
Acenaphthylene	2 : 3-6 : 7-Dibenzophenanthrene	Methyl ethyl ketone
Acetaldehyde	1 : 2-3 : 4-Dibenzopyrene	2-Methylfuran
Acetic acid	3 : 4-9 : 10-Dibenzopyrene	Methylmercaptan
Acetone	Diethyl ketone	2-Methylnaphthalene
Acetylene	Diethylene glycol	3-Methylpyrene
Acrolein	2 : 5-Dimethylfuran	*N*-Methylpyrrolidine
Aluminium	2 : 3-Dimethylpyrrole	Myosmine
Ammonia	2 : 4-Dimethylpyrrole	Naphthalene
Anabasine	3 : 4-Dimethylpyrrole	1-Naphthol
Anodmin	Dipalmityl ketone	2-Naphthol
Anthanthrene	Dipropyl ketone	Naphtho-3 : 4-pyrene
Anthracene	Ethane	Nicoteine
Arsenic	Ethyl mercaptan	Nicotelline
Azulene	Ethylamine	Nicotimine
Benzaldehyde	Ethylene	Nicotinamide
1 : 2-Benzanthracene	Ethylene glycol	Nicotine
Benzene	Fluoranthene	*iso*Nicotine
3 : 4-Benzofluoranthene	Fluorene	Nicotinic acid
1 : 2-Benzonaphthacene	Formaldehyde	Nicotoine
Benzoic acid	Formic acid	Nicotyrine
1 : 12-Benzoperylene	Fumaric acid	Nitric oxide
3 : 4-Benzopyrene	Furan	Nitrogen
Benzyl alcohol	Furfural	Nonylic acid
Butadiene	*d*-Glucopyranose	Nornicotine
Butaldehyde	Glutamic acid	Obeline
Butane	Glutamine	Oxygen
Butene	Glycerol	Phenanthrene
Butylene	Guaiacol	Phenol
Butyric acid	Hentriacontane	Picoline
*iso*Butyric acid	Heptylic acid	Poikiline
Calcium	Hexoic acid	Potassium
Capric acid	Hydrogen cyanide	Propane
Caproic acid	Hydrogen sulphide	Propionaldehyde
Capronic acid	Hydroxy-3 : 4-benzopyrene	Propionic acid
Caprylic acid	Iron	Propylene
Carbon dioxide	Isoprene	Pyridylethylketone
Carbon monoxide	Lathraein	Quinol
Carbonyl sulphide	Lead	Resorcinol
Catechol	Lohitam	α-Socratine
Chromium	Lutidine	β-Socratine
Chrysene	Magnesium	γ-Socratine
Citric acid	Manganese	Sodium
Collidine	Methanol	Solanesol
Copper	Methylamine	Strontium
Coronene	2-Methylanthracene	Succinic acid
o-Cresol	Methylchloride	Titanium
m-Cresol		Toluene
p-Cresol		Valeric acid
Crotonaldehyde		*iso*Valeric acid
Cyanogen		Zinc
*cyclo*Pentadiene		
Diacetyl		

Groups of compounds

Alkylamines	Gaseous sulphocyanides	Paraffins
Condensation products from amines	Higher hydrocarbons	Phenols
Condensation products from ketones	Higher ketones	Pyridine bases
Cyanides	Higher unsaturated compounds	Thiocyanates
Ethereal oils	Hydroxy acids	Unsaturated hydrocarbons
Fatty acids	Nitrates	Volatile acids
		Volatile bases
		Waxes

TABLE IV. CARCINOGENS IN TOBACCO SMOKE

Arsenious oxide (As_2O_3)
1 : 2-Benzanthracene*
1 : 12-Benzoperylene*
3 : 4-Benzopyrene*
Chrysene
5 : 6-*cyclo*Penteno-1 : 2-benzanthracene
6 : 7-*cyclo*Penteno-1 : 2-benzanthracene
1 : 2-5 : 6-Dibenzanthracene
3 : 4-9 : 10-Dibenzopyrene
3-Methylpyrene*

* Identified by workers at the Sir John Cass College

These data are taken from *Survey of compounds which have been tested for carcinogenic activity*, 2nd ed., 1951, by J. L. Hartwell (*Publ. Hlth Serv. Publ., Wash.* No. 149); from material collected for the 3rd ed. of this book, made available by the kindness of M. B. Shimkin; and from data given by Bonnet & Neukomm (1956).

dihydroxybenzenes were also found and determined. Other evidence shows that guaiacol and possibly the two other monoethers of the dihydroxy compounds are present.

ii. *Polycyclic aromatic hydrocarbons* were first shown to be present in cigarette smoke several years ago (Cooper & Lindsey, 1953b; Cooper, Lindsey & Waller, 1954; Commins, Cooper & Lindsey, 1954; Cooper & Lindsey, 1955); and later were detected in pipe and cigar smoke (Gilbert & Lindsey, 1956a, 1956b; Campbell & Lindsey, 1957).

As the suggestion was made that these hydrocarbons in cigarette smoke might be derived from the paper, paper " cigarettes " made entirely from cigarette paper were smoked and the smoke analysed (Cooper, Gilbert & Lindsey, 1955). The analytical results (Table V) show that the yield of hydrocarbons from cigarettes made of paper is similar to that from cigarettes made of tobacco, hence those formed from the paper making up about 4% of an ordinary cigarette are of no importance.

4. Analytical Techniques

The techniques for the detection and determination of hydrocarbons in microgram amounts (alumina chromatography and ultra-violet spectrophotometry) developed during the last seven years at the Sir John Cass College were employed originally for industrial effluents (Wedgwood & Cooper, 1951, 1953, 1954, 1956) but were found of value in examination of soot, air-solids and smoke (Cooper & Lindsey, 1953a; Cooper, 1953; Cooper, 1954; Gilbert & Lindsey, 1955; Waller & Lawther, 1955; Commins, Waller & Lawther, 1955; Campbell & Kreyberg, 1956; Campbell & Clemmesen, 1956). The identification depends upon chromatographic sequences and the recognition of the characteristic absorption peaks in the spectra, additional confirmation being provided by the use of displacement carriers, fluorescence spectroscopy and addition of internal standards. More recently the identities have been confirmed by bromination and the identification of brominated products by spectrophotometry (Goldup, Cooper & Lindsey, 1955).

5. Methods of Smoking

The three methods of smoking (cigarette, cigar, pipe) involve differences especially in: (i) the nature of the tobacco

TABLE V. POLYCYCLIC HYDROCARBONS IN CIGARETTE AND PIPE SMOKE (Campbell & Lindsey, 1956)

A: cyclohexane extract of unsmoked material
B: smoke from materials after extraction
C: smoke from unextracted normal materials

Source of smoke		Acenaphthylene (μg./100 g. material)	Phenanthrene (μg./100 g. material)	Anthracene (μg./100 g. material)	Pyrene (μg./100 g. material)	Fluoranthene (μg./100 g. material)	3 : 4-Benzopyrene (μg./100 g. material)
Cigarettes – –	A	—	—	0.1	10.3	10.6	0.25
	B	0.3	13.0	2.2	3.3	2.3	0.4
	C	5.0	—	10.9	12.5	—	0.9
Paper cigarettes –	A	—	—	0.1	1.4	2.1	0.1
	B	3.3	10.9	4.0	9.2	8.6	1.4
	C	8.3	4.3	16.9	17.0	16.9	4.0
Pipe tobacco (light)*	A	—	—	0.3	19.5	12.6	0.5
	B	2.0	80.0	58.0	40.0	15.0	3.0
	C	29.1	297.0	110.0	75.5	14.7	8.5
Pipe tobacco (dark)*	A	—	—	—	78.0	77.0	5.4
	B	8.9	840.0	88.5	72.0	57.0	7.0
	C	596.0	665.0	198.0	122.0	167.0	11.3
Herbal pipe mixture*	A	—	—	2.0	12.0	15.8	0.3
	B	8.2	180.0	55.0	57.0	21.0	13.0
	C	136.0	104.2	162.0	55.3	104.5	6.5
Herbal cigarette mixture*	A	—	—	0.5	20.5	21.5	1.4
	B	6.5	400.0	81.0	45.0	58.0	6.2
	C	87.0	240.0	7.8	62.9	37.1	8.9

* Smoked in pipe

The results given in lines marked C show that much greater amounts of hydrocarbons are produced from pipe tobacco smoked in a pipe than from cigarettes.

smoked, and the method by which it was cured; (ii) the temperatures produced, and the conditions for oxidation, in smoking; (iii) the filtration of the smoke through unsmoked material (and through the lumen of the pipe); (iv) the relation between the amounts of hydrocarbons produced by pyrolysis, and destroyed by combustion; (v) the amounts of hydrocarbon already present in the unsmoked material; (vi) the practice of inhalation.

The rate of combustion of tobacco may not vary a great deal. The paper of cigarettes makes very little difference.

6. Smoke of Cigarettes, Cigars and Pipes

A comparison of the smoke from cigarettes, cigars and pipes has recently been made from the point of view of content of polycyclic aromatic hydrocarbons (Campbell & Lindsey, 1957), and the results are given in Table VI. The figures shown in Tables V and VI present information upon the processes concerned in the various methods of smoking.

7. Incidence of Cancer of the Lung among Smokers

The larger amounts of 3:4-benzopyrene in the smoke of cigars and pipes are of interest in connexion with the much lower incidence of cancer of the lung among those who smoke tobacco in these forms (see Table VII). Factors which may be considered here are: (i) the alkaline smoke of cigars and pipes, unlike the acid smoke of cigarettes, is not inhaled; (ii) the temperatures reached in cigarettes are higher than those attained in cigars and pipes, and the conditions are more favourable for oxidation; (iii) there appear to be no data as yet about the comparative sizes of the particles or droplets in such smokes, upon which their penetration into the air passages may depend.

TABLE VI. SOME CONSTITUENTS OF MAINSTREAM SMOKE OF CIGARETTES, CIGARS AND PIPES

Constituent	Smoke from cigarettes (μg./100 g. tobacco)	Smoke from cigars (μg./100 g. tobacco)	Smoke from pipes (μg./100 g. tobacco)*
Acenaphthylene	5.0	1.6	29.1
Anthracene –	10.9	11.9	110.0
3 : 4-Benzopyrene	0.9	3.4	8.5
Pyrene – –	12.5	17.6	75.5

* The tobacco used for these experiments was a light pipe tobacco.

TABLE VII. INCIDENCE OF CANCER OF THE LUNG AMONG SMOKERS OF CIGARETTES, CIGARS AND PIPES (Cuyler Hammond, 1956, personal communication)

Type of smoker	All cases reported		Cases with well-established diagnosis, excluding adenocarcinoma	
	Number of deaths	Annual death-rate per 100,000	Number of deaths	Annual death-rate per 100,000
Non-smoker –	9	14.0	2	3.1
Occasional –	2	8.4	2	8.4
Cigar only –	5	16.5	4	13.3
Pipe only –	9	35.2	6	24.1
Cigarette only	151	141.6	97	87.2
Cigarette and other – –	69	81.9	50	57.7
Total –	245	—	161	—

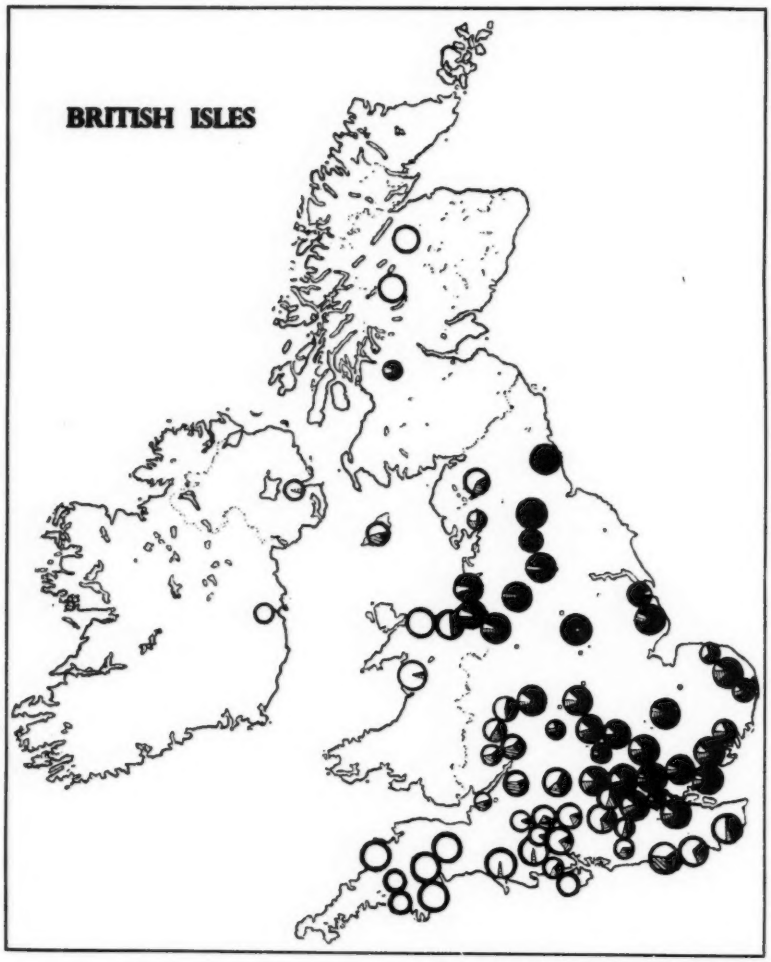
Death-rates standardized for white male population of the USA, 1953.

8. Atmospheric Pollution

Atmospheric pollution in relation to cancer of the lung has been the subject of a good deal of confused thinking, and recently has acquired a spurious importance from the desire to exculpate tobacco. The notes below represent an attempt to lessen this confusion and do not claim to establish any final conclusions.

By about 1840 the smoky atmosphere of the Industrial Revolution of the 18th and 19th centuries in England had produced a change in coloration to black in about 70 species of moths (Kettlewell, 1955a, 1955b, 1955c). The story of the moths is by no means a wholly academic one as it provides quite independent evidence on the subject of atmospheric pollution. Dr Kettlewell's map (fig. 1) shows: (i) a correlation between industrial areas and a high percentage of the black

FIG. 1. FREQUENCY MAP OF *BISTON BETULARIA L.*, THE PEPPERED MOTH, AND ITS TWO MELANIC FORMS (*F. CARBONARIA* AND *F. INSULARIA*) IN BRITAIN



Photograph kindly supplied by H. B. D. Kettlewell

typical form

F. carbonaria Jdn

F. insularia Th. Mieg

The map shows the results of more than 20,000 records, from 83 centres, from 1953 to 1957.

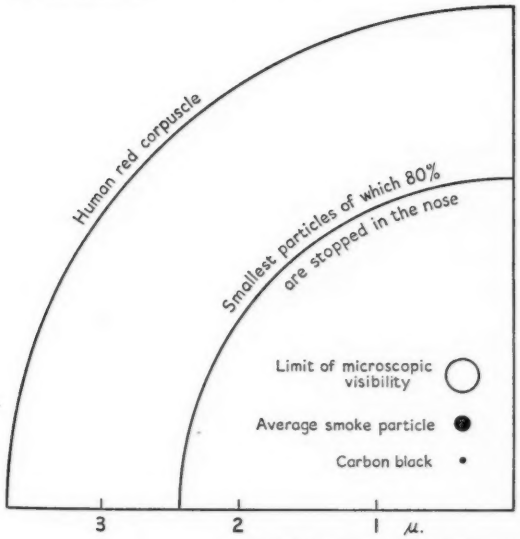
form of moth; (ii) absence of melanic moths from pollution-free areas (North Scotland, Devon and Cornwall); (iii) a high proportion of melanic moths to the east of all industrial areas extending as far as the whole of the east coast. This probably reflects indirect effects of the drift of pollution due to the prevailing south-westerly wind.

During the present century, certain factors tending to reduce atmospheric pollution have been active, namely, the more economical combustion of smoke-producing fuels, and the replacement of these fuels by coal-gas and electricity; this second factor is of especial importance in relation to domestic heating and cooking, as the coal-fired household grate and range are the chief source of atmospheric smoke. There is no doubt that remedial measures can be very effectual: for example, Pittsburgh (USA) has been transformed from one of the dirtiest cities in the world to one of the cleanest. These factors tending to reduce atmospheric smoke are the exact opposite of anything which could account for the sudden increase in cancer of the lung in Great Britain in the period 1920–25, which increase has continued until the present day.

Any estimate of the total intake by man of any constituent common to coal smoke and to cigarette smoke (e.g. arsenic, 3 : 4-benzopyrene), though we have now sufficient data to make such an estimate fairly accurate arithmetically, is misleading in relation to the aetiology of cancer of the lung. Tobacco smoke reaches the respiratory tract solely through the mouth, whereas most of the air which we inspire passes through the very efficient nasal filter which arrests 80% of particles larger than 2.5 μ. (fig. 2).

This consideration is of great importance in relation to the following observations.

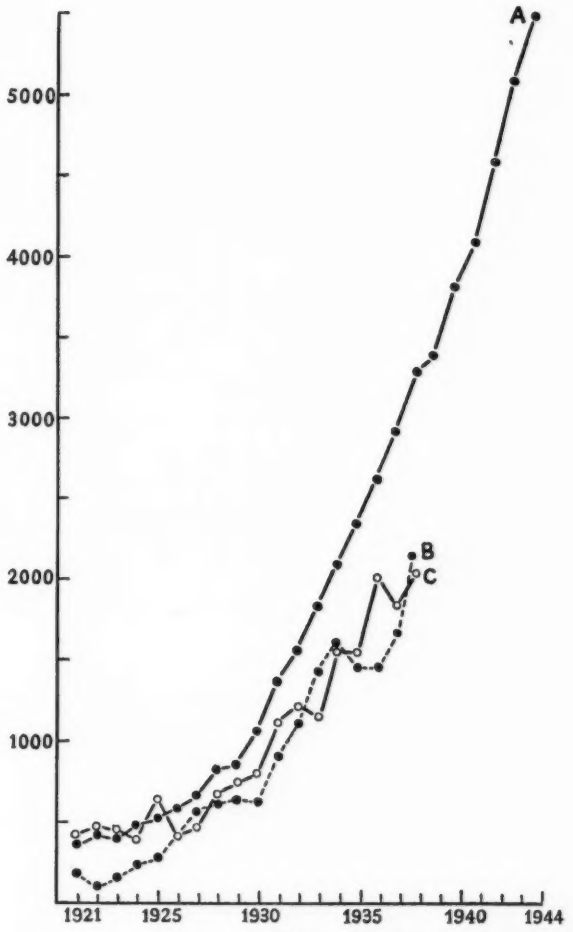
FIG. 2. ARREST OF SMOKE PARTICLES, AND THEIR SIZE IN RELATION TO SIZE OF HUMAN RED CORPUSCLE



Blacklock, Kennaway, Lewis & Urquhart (1954) by permission of British Journal of Cancer

Carbon black indicates particle within the range 10 mμ. to 17 mμ. giving maximum adsorption of 3 : 4-benzopyrene.

FIG. 3. DEATHS FROM CANCER OF THE LUNG AMONG MALES IN ENGLAND AND WALES, FROM 1921 TO 1944



Kennaway & Kennaway (1947) by permission of British Journal of Cancer

Abscissae: year

Ordinates: number of deaths

Curve A: deaths among the general population, from 1921 to 1944
Curve B: deaths among coal-miners
Curve C: deaths among agricultural workers
} ×20, from 1921 to 1938

i. Experiments in which animals (e.g. mice, hamsters) are kept in atmospheres containing tobacco smoke are misleading in relation to cancer of the lung in man, because these animals do not keep their mouths open for the purposes of the experiment and, in many rodents, the structure of the turbinate bones is much more complex than it is in man.

ii. The comparison of data from different countries, and even from different areas of the same country, is made difficult by the fact that atmospheric pollution is not of a uniform character. Thus one could select two towns, say, in Norway and in England, where a given volume of air contains

TABLE VIII. ORGANIC CONSTITUENTS OF TOWN AIR

Individual compounds	
Acenaphthene	Ethane
Acenaphthylene	Ethylene
Acetylene	Fluoranthene
Acrolein	Fluorene
Anthanthrene	Formaldehyde
Anthracene	*n*-Hexane
Azulene	Methane
1 : 2-Benzanthracene*	2-Methylnaphthalene
Benzene*	3-Methylpyrene
1 : 2-Benzopyrene	Naphthalene
3 : 4-Benzopyrene*	*n*-Pentane
n-Butane	*iso*Pentane
*iso*Butane	2 : 2-Dimethylpropane
Chrysene*	Perylene
Coronene	Phenanthrene
1 : 2-9 : 10-Dibenzonaphthacene	Pyrene
4 : 9-Dimethylpyrene	Toluene
Groups of compounds	
Acids	Ketones
Alcohols	Peroxides
Aldehydes	Phenols
Epoxides*	Pyridine compounds
Gasoline	Sulphur compounds
Glycols	Tar acids
Hydrocarbons (gaseous)	Xylenes
Hydrocarbons (unsaturated)	

* Indicates carcinogenic compounds

the same amount of suspended matter; but it may not be of the same composition and certainly may not contain the same amount of the same carcinogens, or of cocarcinogen. Our methods of estimation measure blackness, which is affected by substances which are not black—road dust, for example. Thus the suspended matter of Los Angeles is said to contain large amounts of gasoline, while the corresponding material in Reykjavík is probably composed largely of road dust.

Campbell & Stocks (1955) conclude that atmospheric pollution makes a very distinct contribution to the incidence of cancer of the lung in the Liverpool area. Many other investigators do not agree with this conclusion, but no evidence that is not based upon the particular form of suspended matter present in Liverpool can be considered a valid basis for disagreement.

That the increase of cancer of the lung of recent years is of occupational or industrial origin (Hueper, 1955) was shown to be extremely improbable ten years ago (Kennaway & Kennaway, 1947; and fig. 3) by the fact that the number of deaths in two large groups (coal-miners and agricultural workers) in which the incidence was, and is, very low, was increasing at the same rate as in the general population of England and Wales. In the same paper it was pointed out that the number of deaths from cancer of the lung had become so large that, if the increase occurred in any small number of occupations, the incidence in these would have to be extremely severe. In the same paper (fig. 7, p. 285) a graph of deaths from cancer of the lung in carpenters, whose occupation is not especially associated with cancer of the lung, shows a rate of increase similar to that in the general population.

The term " cancer of the lung " includes several different pathological conditions distinguished by histological type. We do not know the histological class of cancers of the lung, if any, induced by town smoke. Kreyberg (1956) has made the first comprehensive study of the histological type of all the cancers of the lung occurring in a whole population, namely in parts of Norway during the period 1925–55.

The various possibilities of carcinogenic and cocarcinogenic action in such material as the suspended matter of the air of a town are considerable. Thus, in any one case there might be: a single carcinogen; more than one carcinogen, the effects of which might summate; one or more cocarcinogens; solvents of carcinogens (Cooper, 1954); particles with various powers of adsorbing carcinogens and cocarcinogens (Falk & Steiner, 1952); and all these may vary with the time of year and the locality.

9. Composition of Town Air

Many materials are found in town air in locally high concentrations and are often due to specialized activities: for example, cement dust in the vicinity of building operations, or petroleum vapours in streets congested with traffic. The universal characteristic of urban air is the presence of partial combustion products and unburnt fuels. In Table VIII are listed organic compounds detected in urban air, with a subsidiary list of classes of compounds that have been recognized without identification of all individuals of the class.

TABLE IX. CANCER OF THE TRACHEA, BRONCHUS AND LUNG, IN ENGLAND AND WALES FROM 1943 TO 1954 (figures taken from Registrar-General's Statistical Reviews for 1953 and 1954, Tables, Part I, Medical)

	Males			Females		
	Deaths	Rate per million living	Increase in mean rate	Deaths	Rate per million living	Increase in mean rate
1943	4,943	303		1,166	54	
1944	5,331	329		1,237	57	
1945	5,805	354		1,356	62	
1946	6,607	355		1,437	65	
1947	7,581	387		1,623	73	
1948	8,403	413		1,759	79	
Mean of first 6 years	6,445	375.0		1,430	65.0	
1949	9,130	444		1,845	82	
1950	10,254	485		1,987	87	
1951	11,166	530		2,081	91	
1952	11,981	568		2,237	98	
1953	12,881	607		2,251	98	
1954	13,995	658		2,336	101	
Mean of second 6 years	11,568	548.7	46.3%	2,123	92.8	42.8%

Ratio of mean rates, female to male: first 6 years, 1:5.8; second 6 years, 1:5.9.

10. Geographical Incidence of Cancer of the Lung

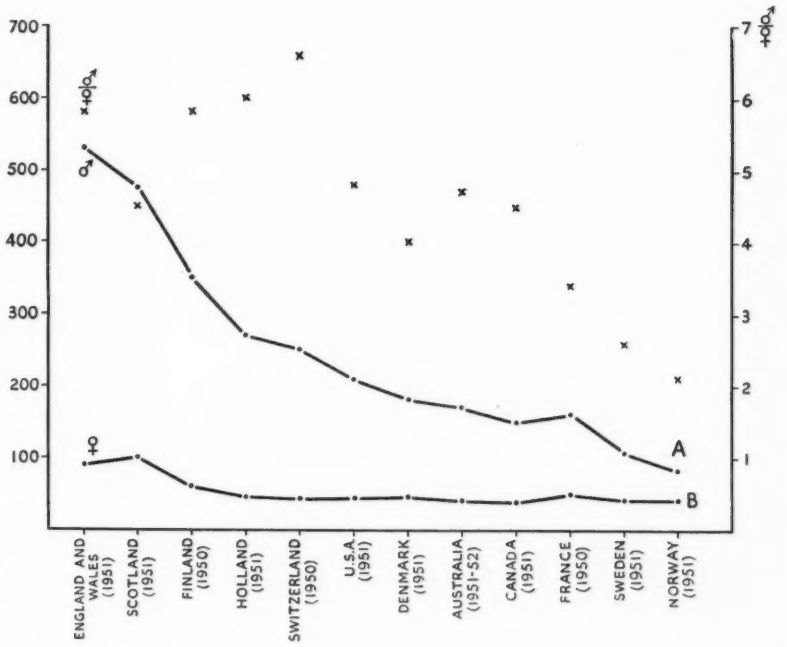
Figure 4 displays data given by Doll (1955) which show that a high incidence of lung cancer is due to some factor affecting males more than females. This does not of course exclude atmospheric pollution, in view of our very limited knowledge of sexual factors in cancer, but it is much more probably due to some factor such as smoking which affects males especially.

The comparison of fig. 4 with the data given in Table IX (Kennaway, 1957) is instructive. Within the boundaries of any one country a very considerable increase in the total incidence of lung cancer can take place without affecting the sex ratio. A similar constancy of sex ratio is shown by cancer of the stomach in relation to social class, which has been maintained in England and Wales during 30 years (Kennaway, unpublished work).

11. Conclusion

This summary of existing knowledge of possible exogenous factors in the causation of lung cancer demonstrates the need for fuller chemical investigation of tobacco, tobacco smoke and town air.

FIG. 4. GEOGRAPHICAL INCIDENCE OF CANCER OF THE LUNG, BASED ON FIGURES OF DOLL (1955)



Abscissae: country

Ordinates:

Left-hand: crude death-rate per million
Right-hand: ratio of death-rate in males to death-rate in females

Curve A: incidence among males
Curve B: incidence among females
× × × ratio of males to females

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CARCINOGENICITY OF MINERAL OIL FRACTIONS

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1 Introduction
2 Fractionation and biological experiments
3 Chemical studies
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1. Introduction

The high incidence of skin cancer among cotton spinners has been known for many years (Southam & Wilson, 1922; Southam, 1928; Henry & Irvine, 1936), and the carcinogenicity of the lubricating oils used for the mules has been demonstrated by experiments on mice (Leitch, 1922, 1924; Twort & Ing, 1928). The carcinogenicity of mineral oils is by no means confined to the spindle oil fractions. Extensive series of animal experiments (Twort & Fulton, 1929; Lyth, 1933; Twort & Twort, 1930, 1933; Twort, 1934; Woodhouse & Irwin, 1950) have shown activity in a wide range of fractions and extracts, and recently Cruickshank & Squire (1950) have ascribed the occurrence of skin cancer among employees in the engineering industry in the Midland counties of England to the cutting oils used with the machines. Further evidence for the potentially carcinogenic activity of cutting oils has been adduced by Cruickshank & Gourevitch (1952), by Mastromatteo (1955) and by Gilman & Vesselinovitch (1955). Some of this work has been reviewed by Brockbank (1941a, 1941b, 1942), Merewether (1944), Henry (1946, 1947, 1950) and by Auld (1950). There can be little doubt, therefore, that the wide-spread use, in industry, of high-boiling mineral oil fractions and extracts constitutes an important potential hazard to health. Table I, showing the incidence of epithelioma ascribable to mineral oil during recent years, is taken from returns of the Chief Inspector of Factories:

TABLE I. EPITHELIOMATOUS ULCERATION WITH FATAL CASES

1950	1951	1952	1953	1954	1955
28 (8)	19 (1)	34 (1)	60 (42)	30 (9)	48 (13)

Fatal cases are indicated in brackets.

A similar carcinogenic industrial material is coal-tar, and from this a potent carcinogenic constituent has been isolated and shown to be the fused ring aromatic hydrocarbon 3:4-benzopyrene (Cook & Hewett, 1933; Cook, Hewett & Hieger, 1933). Other carcinogens are present in coal-tar, as fractions free from benzopyrene—some boiling as low as 200–250° C.—have also been found to have powerful activity (Berenblum & Schoental, 1947; Poel & Kammer, 1957; Lijinsky, Saffiotti & Shubik, 1957). Shale oil (Leitch, 1922; Twort & Lyth, 1933) is another industrial product whose carcinogenic activity has been ascribed, at least in part, to its content of 3:4-benzopyrene (Berenblum & Schoental, 1943; Cahnmann, 1955).

More recently, extensive studies of the high-boiling fractions of cracked mineral oils carried out in the United States of America, notably at the Postgraduate Medical School, New York University, at the Sloan-Kettering Institute, New Jersey, and at the Kettering Laboratory of the College of Medicine, Cincinnati, have shown that these products also, in many cases, have considerable carcinogenic activity (Smith, Sunderland & Sugiura, 1951; Sunderland, Smith & Sugiura, 1951; Fischer, Priestley, Eby, Wanless & Rehner, 1951); and Tye, Graf & Horton (1955) have succeeded in isolating 3:4-benzopyrene from one fraction of a cracked oil.

By contrast, no pure carcinogen has ever been isolated from uncracked distillates of crude petroleum, and the nature of the substances responsible for their activity, and even the class of compounds to which they belong, are unknown. Attempts have been made to distinguish, by physical means, carcinogenic from non-carcinogenic oils, using a formula based on a relationship involving specific gravity and specific refractivity (Twort, 1934), but this has been found unsatisfactory (Auld, 1938; Irwin & Goodman, 1946), and animal experiments furnish the only available means of assessing the carcinogenicity of mineral oil fractions. A fundamental investigation of the problem, which has as its object the separation and characterization of the carcinogenic fractions in petroleum and, if possible, the identification of the substances responsible for their activity, is now being carried out under the aegis of the Medical Research Council, and with the collaboration of the Institute of Petroleum's Research Group No. 2. It is with these developments that this account is primarily concerned.

2. Fractionation and Biological Experiments

Three representative crude oils have been chosen for study, from fields in Kuwait, Lagunillas and Oklahoma. A preliminary separation of each by distillation was carried out at the research centre in Thornton, Cheshire, of the Shell Development and Marketing Company, and later, an extensive series of subfractionations and solvent extractions was performed under the supervision of Professor F. Morton in the Department of Chemical Engineering of the University of Birmingham. Since it is known that very high temperatures may convert mineral oil constituents into carcinogenic substances (Smith *et al.* 1951), the conditions of distillation throughout the work have been carefully controlled so as to eliminate any risk of producing carcinogens by cracking. By working under reduced pressure and, if necessary, by introducing steam, steps have been taken to maintain the temperature of the boiling liquid below 250° C. during distillations,

and thus to ensure that any compounds isolated are reasonably certainly present in the original crude oil.

The distribution of the carcinogens at each stage in the fractionation has been followed by skin-painting experiments on mice and rabbits. It was apparent from earlier work that more reliable results would be likely to be obtained by using more than one species of animal in the tests (Cruickshank & Squire, 1950; Hieger & Woodhouse, 1952; Smith *et al.* 1951; Shubik & Saffiotti, 1954; Hueper, 1956). In the rabbit experiments, random applications were made on a number of sites on each animal (Hieger & Woodhouse, 1952). The biological tests were carried out mainly in the Cancer Research Laboratories, Birmingham University. Many of the tests were made also at the Chester Beatty Research Institute, London, and the duplicate results confirmed the close reproducibility in quantitative terms of such painting experiments. Furthermore, by including a standard carcinogen preparation, results obtained in successive years could be reliably compared with one another. In the event, the oils were found to have a very low activity for mouse skin, but in many instances they displayed a moderate degree of carcinogenicity in the rabbit tests, and statistical analysis of the results by Dr J. O. Irwin of the Medical Research Council's Statistical Research Unit gave a reasonably definite basis for selecting the samples for continued investigation.

Preliminary distillation of the three crude oils at the Thornton Research Centre showed that, in each case, the carcinogens were associated with the fraction boiling in the range 300–400° C. For technical reasons, most of our attention, so far, has been devoted to the active fraction of the Kuwait oil, and this has been subjected to a series of operations designed to concentrate further the carcinogenic constituents. This elaborate and time-consuming series of operations, carried out by Professor Morton and his collaborators and involving specialized skills and otherwise inaccessible equipment, does not lend itself to summary, but it formed an essential prelude to the detailed biological and chemical studies on the fractions. The material was fractionally distilled in Professor Morton's Department and collected in 2.5° C. fractions (Carruthers, Cerigo, King, Morton, Pell & Sagarra, 1955). It was found that most of the activity was contained in the portion boiling between 350° and 400° C., but there was no significant difference in the fractions within this range. Evidently, however, the activity is associated with the aromatic constituents, for on extraction of the fractions with aqueous acetone or with furfural, solvents which are known to be selective for the aromatic components of mineral oils, almost the whole of the activity passed into the extracts, although these amounted to only about 20% of the original fraction. The insoluble material was very feebly active. A similar result has been achieved by extraction of fractions produced from the Lagunillas oil, and it is also in line with experiments by Woodhouse (Woodhouse & Irwin, 1950), who showed that a number of commercial extracts of mineral oil, rich in aromatic components, were carcinogenic to mouse skin. Reaction of the extracts with maleic anhydride removed a fraction containing anthracene derivatives (Carruthers, 1956), which was relatively inactive. Treatment of the remaining part of the extracts with picric acid in ethanol (Carruthers & Douglas, 1957) achieved a further partial separation of the carcinogens. Both the materials recovered from the crystalline picrates and from the mother liquors were active, but the main activity was in the latter, and subsequent investigations have used this material. Preliminary experiments have now shown that, after chromatography on alumina, the carcinogens are largely concentrated in the fractions eluted with petroleum ether and with benzene, and work is now proceeding in Professor Morton's new Department in Manchester to accumulate larger quantities of these fractions for further investigation.

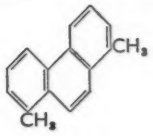
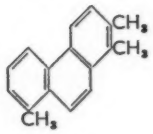
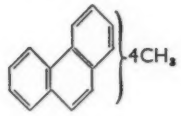
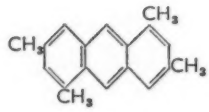
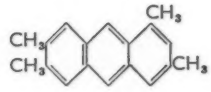
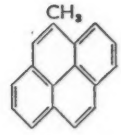
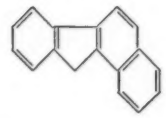
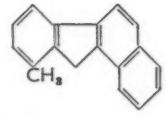
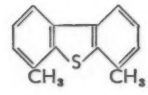
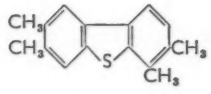
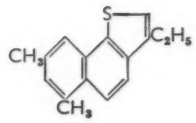
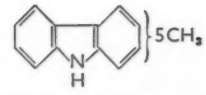
3. Chemical Studies

To follow up the biological and fractionation experiments described above, many of the fractions of the Kuwait oil have been examined chemically, with the object ultimately of identifying the substances responsible for their carcinogenic activity. Working on the assumption that the carcinogenic ingredients of mineral oils may be of similar type to those in coal-tar and shale oil, attention has been centred mainly on the polycyclic aromatic materials in the oil. We have felt justified in this, for not only do the most potent carcinogens known belong to this class of compound, but also it has been our experience that all the carcinogenic mineral oils we have examined contained polycyclic aromatic fractions, and, furthermore, the carcinogenic activity appears to be associated with the aromatic fractions.

Until lately, very little attention had been paid to the constituents of high-boiling fractions of mineral oils, and the most complex aromatic hydrocarbons found in such a source were a number of trimethylnaphthalenes, isolated from a Roumanian oil by Gavăt & Irimescu (1941, 1942). By application of chromatography on alumina and by crystallization of molecular complexes with picric acid, we have been able to isolate and identify a considerable number of pure substances from fractions of the Kuwait oil (see Table II) (Carruthers & Cook, 1954; Carruthers, 1956; Carruthers & Douglas, 1957). Several of these substances are polycyclic aromatic hydrocarbons of a degree of complexity approaching that of the known polycyclic aromatic carcinogens, and it is conceivable that they may be weakly carcinogenic and thus contribute to the total activity of the oil fractions. With this in mind, some of these compounds are now undergoing biological test. An interesting feature of the Kuwait oil is the very high sulphur content, and spectrographic evidence appears to indicate that many of the carcinogenic fractions contain considerable quantities of dibenzothiophen derivatives and, possibly, other condensed polycyclic thiophen compounds. A number of compounds of these types has been isolated from the picrate-forming fractions of the Kuwait oil and, in three cases, identified (see Table II) (Carruthers, 1955; Carruthers & Douglas, unpublished work). Of these, 1:2:6:7-tetramethyldibenzothiophen and 3-ethyl-6:8-dimethylnaphtho-(1:2-b)-thiophen had not previously been described in the literature; whereas 1:8-dimethyldibenzothiophen has recently been isolated from a coal-tar fraction (Kruber & Raeithel, 1954). The occurrence of condensed polycyclic thiophen derivatives in fractions of the Kuwait oil is interesting in view of the observation that a number of tetracyclic compounds of this type have powerful carcinogenic activity (Hershberg & Fieser, 1941; Robinson, 1946; British Empire Cancer Campaign, 1946; Waravdekar & Ranadive, 1957).

The extraordinary complexity of high-boiling fractions of mineral oils has presented considerable difficulties during this investigation. It is evident that a clear-cut separation of the carcinogenic and non-carcinogenic fractions of the oil will be achieved only by the application of the most modern

TABLE II. SUBSTANCES ISOLATED FROM FRACTIONS OF KUWAIT MINERAL OIL

Isolated by chromatography of maleic anhydride extracts		Isolated by crystallization of picrates and chromatography on alumina	
2:6-Dimethylanthracene		1:8-Dimethylphenanthrene	
2:7-Dimethylanthracene		1:2:8-Trimethylphenanthrene	
2:3:6-Trimethylanthracene		Tetramethylphenanthrene, m.p. 99–100° C.	
1:3:5:7-Tetramethylanthracene		Tetramethylfluorene, m.p. 198–199° C.	
1:3:6:7-Tetramethylanthracene		1-Methylpyrene	
2:3:6:7-Tetramethylanthracene		1:2-Benzofluorene	
Four unidentified tri- or tetra-methylanthracenes, m.p. 67–69° C., 108–110° C., 169–170° C., 224–225° C.		8-Methyl-1:2-benzofluorene	
		1:8-Dimethyldibenzothiophen	
		1:2:6:7-Tetramethyldibenzo-thiophen	
		3-Ethyl-6:8-dimethylnaphtho-(1:2-b)-thiophen	
		Pentamethylcarbazole, m.p. 184–185° C.	

techniques. It is true, also, that spectroscopic examination of the active fractions from the latest stages of the separation indicates that they are still very complex mixtures. Nevertheless, the results of the chemical work indicate that it is possible to isolate some of the components of these mixtures in a pure state, and justify the hope that the ingredients responsible for their carcinogenic activity may ultimately be isolated and identified.

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OCCUPATIONAL CARCINOGENESIS

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1 Asbestos
2 Chromium
3 Nickel
4 Haematite
5 Coal and graphite
6 Gas and coke
7 Pitch, tar, soot and mineral oil
8 Aromatic amines
References

Interest in this subject rests not only on the obvious implications for industrial hygiene and safety but also on the suspicion that there may be many more carcinogenic influences at work than are at present known or suspected, and that non-occupational cancer may also be related to some exogenous causes. Moreover, the recognition of occupational carcinogens must lead to fruitful experimental study not only to sustain the clinical or statistical contention but to seek some underlying unity among the diverse materials or their effects which can evoke the cellular neoplastic reaction. The agreement that a particular material is an occupational carcinogen requires ideally that it shall be shown, by long collection and analysis of occupational and clinical data, that the incidence of the disease among those exposed to the suspected agent so far exceeds the incidence of its non-occupational counterpart that there can be no reasonable doubt of the nature of the carcinogenic agent or its precursor, or of the process in which it resides. When, as is usually the case, it is necessary to build on retrospective data, death-certificates, befogged occupational histories, mobility of workers, multiple exposures, poor or no factory records and inadequate information on past environmental conditions, the difficulties are much increased in reaching any clear conclusions. The present brief consideration will be restricted to those materials which were more or less summarily discussed in volume 3 of Merewether's *Industrial medicine and hygiene* (Goldblatt & Goldblatt, 1956).

1. Asbestos

The peculiar association of carcinoma of the lung with asbestosis, first presented by Merewether (1949) and Gloyne (1936, 1951) with strongly suggestive statistics, finds confirmation in the Annual Report of the Chief Inspector of Factories and Workshops for 1955. For the period 1924–55, 22% of 222 men and 12% of 143 women showed combined asbestosis and lung cancer, indicating a rising incidence of the association as compared with that reported in 1947 and in 1954. So high an association does not occur in silicosis (see Goldblatt & Goldblatt, 1956; Gloyne, 1951; Meiklejohn, 1956). Werber (1952) states that carcinoma of the lung follows epithelial metaplasia in 7–17% of cases of asbestosis, with a latent period of 1½–20 years. Doll (1955) analysed the post-mortem findings in 105 workers in a single large asbestos works, whose deaths occurred consecutively. Of these 75 had asbestosis, and of them 15 had associated lung cancer, whereas of the remaining 30 without asbestosis 3 had the tumour. All the 15 with asbestosis cancer had worked for periods of 9–23 years in the industry before 1933, when the 1931 Asbestos Industry Regulations became effective in the factory, but the association was not recorded in any person entering the industry for the first time between 1923 and 1953. In the mortality data of 113 men who had worked at least 20 years in the factory, Doll found 11 death-certificates with asbestosis and lung cancer, whereas there were none of lung cancer without asbestosis. This incidence is some ten times that expected from the England and Wales mortality data for lung cancer. Observations published over 20 years ago in South Africa by the Miners' Phthisis Medical Bureau, cited by Doll (1953), showed that the incidence of primary lung cancer in silicotic miners was 0.7%, for non-silicotic miners 0.7% and for European male surface workers 0.9%. Agreement on the relation between asbestosis and lung cancer was reached at the international Symposium on the Endemiology of Lung Cancer (1953).

Wyers (1949) reported the cell types to be oat cell, columnar and endothelial (pleura), the first two being the most frequent. According to Hunter (1955) the carcinoma usually appears many years after exposure to asbestos has ceased. This is not borne out by the data in Doll's paper, where the periods from last exposure to death in the 15 cases with the combined diseases are given as less than 1 year in 6 cases, 1 year in 3, 3 years in 3, 8 years in 2 and 13 years in one.

The induction time is probably very long, inasmuch as the tumours seem always to be of high malignancy, with only a short time between commencement of tumour process and death.

As to cause and mechanism, Meiklejohn (1956) and Chiurco (1956) speak of irritant factors, whereas Bonser, Faulds & Stewart (1955) suggest that the common factor in the exposure of the asbestos textile worker and the haematite miner (see section 4 below) is silica and that in both types of lung cancer the fibrosis precedes the malignant process. They conceive the fibrous asbestos (probably chrysotile) and the iron oxide as modifying the action of silica, or of the fibrotic process, so that silica acts more as a carcinogen and less as a fibrosing agent.

No experimental evidence is available that asbestos in any form (small or large fibres) can act as a carcinogen, although peribronchiolar fibrosis has been demonstrated in rabbits by King, Clegg & Rae (1946) and others; but if the asbestos is fused and ground no fibrosis is induced even after long inhalation. Physical form and flexibility are evidently determinants, since peribronchiolar fibrosis can be induced also by brucite which is a native, crystalline form of $Mg(OH)_2$ and contains only some 0.9% SiO_2 as silicate (chrysotile contains over 40% SiO_2 as silicate).

It must be noted, also, that three of Doll's patients who had the first exposure after 1939 and total exposures of 2, 9 and 7 years, showed no asbestosis and died in 2, 12 and 11 years after the first exposure, from carcinoma of the lung. Knox & Beattie (1954a) would explain such cases as not having had sufficient time to develop asbestosis, and that the neoplastic change, induced by asbestos as such, by asbestosis

bodies or by some endogenous agents, including decomposition or solution products of asbestosis bodies, may precede the changes necessary in the inhaled material to induce asbestosis proper. In another communication, Knox & Beattie (1954b) confirmed Gloyne's view that with time the number and size of asbestosis bodies and fibres in the lungs diminished, an opinion held previously by the pathologist M. J. Stewart of Leeds. Stewart regarded the fibres and asbestosis bodies as being gradually dissolved, the products inducing the lesions which survive the fibres. Evidence supporting these views as to solubility was obtained with electron microscopy by Champeix & Bouteville (1950).

Knox (1955) is, on the whole, of the opinion that the epithelial changes leading to carcinoma arise from: (i) chronic irritation leading to increased thickening and compression of the alveolar walls and (ii) subsequent slow disintegration of the asbestosis bodies with liberation of something which, acting on the already abnormal epithelium, induces malignancy. The views of Chiurco (1956) apply here as elsewhere, to the effect that, in the interpretation of the occupational precancer and tumour states, it is very important to consider the soil prepared for cancer; the aetiology is multiple, syncarcinogenesis and cocarcinogenesis together with a whole constellation of factors, co-factors and co-causes; these bring about and favour a restless terrain, labile, unstable, dynamic, on which will develop cancerization by the release of factor X.

2. Chromium

In the case of chromium we have an element of which particular compounds are either poisonous or induce chronic ulceration, dermatitis or caustic effects. Chrome holes, nasal septum perforation, chrome dermatitis and chrome burns are not followed by neoplastic change (cf. Legge, 1922). But from 1935 onwards, Continental and American observers presented evidence in support of a lung carcinogen in the chromate industry, in the manufacture of chrome pigments and in the use of zinc and lead chromates. Hueper (1942) thought that the true carcinogen was produced by the action of chromium on some endogenous material. A recent case of bronchogenic carcinoma in an Elektron[1] polisher, described by Asang (1952), showed long retention of insoluble Cr compounds in the lungs (8–10 mg. Cr) even seven years after leaving the work. On the other hand, Miller (1956) states that only those exposed to the mists of water-soluble chromates (Cr^6) develop lung tumours, but, of course, these must undergo reduction to be retained.

In Britain, however, Bidstrup (1951) could find only one case of lung cancer (later fatal) in a radiographic survey of 724 workers in three chromate factories. In a later study of the 723 workers in the six years following the first survey, Bidstrup & Case (1955) found that, of 59 deaths, 12 had been certified as due to cancer of the lung, 9 to tumours of other sites and 38 as due to other causes; in addition 1 other lung-cancer death and 2 living patients with lung cancer were known but not included. Only the lung-cancer cases were found to be significant, being almost four times that expected, all statistically important factors having been allowed for. It is likely that the hazard will appear much higher than this with the passage of years as more and more workers die.

[1] Elektron metal is an alloy of Mg with small amounts of Al, Zn, Si and Mn. The metal objects are dipped in alkaline dichromate to produce a coating of Al chromate and Mg chromate.

As in all occupational cancers the latent or induction period is long, in this series 21 ± 10 years; and the tumours may appear many years after exposure in the industry has ceased.

Nothing is known as to the active agent but it appears to the writer that it is likely to be a trivalent chromium compound (Goldblatt & Goldblatt, 1956).

3. Nickel

In a paper enumerating certain substances which cause cancer in industry, Bonser *et al.* (1955) suggest that nickel may induce cancer of the respiratory tract, but add that the proof that the tumours are industrial in origin is doubtful, and that no experimental proof has been adduced. This is a surprising statement inasmuch as carcinoma of the mucous membranes of the nose and air sinuses, and bronchial carcinoma, are prescribed diseases for the purposes of the National Insurance (Industrial Injuries) Act, 1946, and the nature of the occupation is given as decomposition of a gaseous nickel compound. Manifestly the framers of the prescription had $Ni(CO)_4$ in the Mond process in mind (see Amor, 1939), and left no doubt that they believed the condition to be industrial even if they did not indict nickel metal or even the carbonyl as such. The indictment was of an industrial process and it may be that Bonser and her co-workers imply that nickel, as such, is doubtful as the industrial cause. The problem is presented by Goldblatt & Goldblatt (1956), and the two common theories —the "arsenic theory" and the "metal theory"—are discussed.

The "arsenic theory" (metallic arsenides) is out of favour because: (i) there is no collateral evidence of arsenical affections (dermatitis, skin cancer); (ii) other dusty industries in which sulphuric acid with a high As_2O_3 content has been used for many years are not prone to cases of ethmoid or lung cancer; (iii) heavy exposure to arsenical dusts sufficient to perforate the nasal septum does not induce such cases. Nevertheless Perry (1947) found a raised As content in the lungs, hair and urine of two nickel workers in whom extirpation of the lung had been successfully performed.

The "metal theory" rests perhaps on stronger grounds and, inasmuch as cases of $Ni(CO)_4$ poisoning still occur, we cannot ignore the likelihood of deposition of nickel at susceptible points. Indeed, Hunter (1955) does not discard either nickel or the carbonyl as possible causes. For an account of the removal of nickel after exposure to $Ni(CO)_4$, see Barnes & Denz (1951).

Nickel is not an inert substance; dermatitis occurs frequently following exposure to nickel. Calnan & Wells (1956) patch-tested hundreds of patients and found nickel to be the commonest specific skin sensitizer, nearly always in women (ear-rings, suspenders, spectacles, brassière fastenings, bracelets, etc.). Once established, the sensitivity was usually maintained, and secondary spread was a feature.

Schinz & Uehlinger (1941) and Hueper (1951, 1952) induced tumours in highly artificial conditions, and Løken (1950) found three cases (two more later) of bronchogenic carcinoma in men working on electrolytic refining of nickel (no carbonyl involved) in a single factory; one lung, analysed eight years after cessation of exposure, contained 1 mg./g. nickel. It is, perhaps, significant that the nickel mineral used in most countries consists of a mixture of NiFeS, $CuFeS_2$ and Fe_9S_9, with small amounts of Au, Ag, and Pt.

Cancer of the nose and ethmoid is a rare condition in the

general population. The mean induction time of the occupational tumour is about 23 years. We suggest that the carcinogen enters the ethmoid sinus and that the rapid decomposition of the carbonyl in the tissues leads to the deposition of very finely divided nickel in the thin mucous membrane, which is very closely applied to the bone. Retention would be accentuated by any degree of stenosis at the fine orifices connecting the sinuses to the nasal meatus, say from slight oedema. It is significant that Løken makes no mention of nasal or ethmoid tumours in his five cases, a carbonyl process not being involved. Barnes & Denz (1951) showed that rats exposed to $Ni(CO)_4$ frequently develop severe and extensive fibrosis of the lungs, a reaction evidently due to a stable deposition of nickel or a nickel-protein complex in the alveolar walls. The significance of the mild cases of $Ni(CO)_4$ intoxication in factories may become apparent in later years.

4. Haematite

Faulds & Stewart (1956) have found that, in the six-year period 1948–53, 15% of 89 consecutive necropsies of haematite miners showed cancer of the lung, and that the tumours usually lay in the areas of fibrosis caused by sidero-silicosis; they consider that the latter condition predisposes to carcinoma of the lung. The view of Bonser *et al.* (1955), that a modification of the action of SiO_2 by asbestos and by iron oxide may turn it into a carcinogen, recalls that Kennaway & Kennaway (1947) found no evidence of an increased cancer-rate among workers exposed to the risk of silicosis except in the case of metal-grinders. Turner & Martin (1949) confirmed this by finding that 60% of deaths from cancer in grinders with silicosis are due to cancer of the lung. It may also be recalled that Kettle (1932) found that a thin layer of iron oxide over quartz particles prevents the development of silicosis in animals. But Doll (1953) attributes any increased cancer hazard in grinders to "some other, and more specific, industrial hazard" and not to silica.

5. Coal and Graphite

Kennaway & Kennaway (1953) found a lower incidence of lung cancer in coal-miners than in the general population, thus going further than Gooding (1946) who found no evidence of increased primary lung cancer in pneumoconiotic anthracite-miners. Moreover, it appeared that there was an almost inverse relation between the two conditions. James (1955) casts more light on some selected findings of Stocks (1952) that the relative incidences of lung cancer in the period 1946–49 in the South Wales towns of Merthyr Tydfil (high coal-miner population), Cardiff and Swansea (few coal-miners) were as 77 : 126 : 132, the lower incidence being maintained in the mining area even after correction for the larger population and urbanization of the latter two towns. For 1947–52 James found primary lung cancer at necropsy in 3.3% of 1,827 South Wales coal-miners and in 5.4% of 1,531 non-miners over the age of 21. In both series the frequencies of cell types and of metastases were of the same order. Where massive fibrosis and cancer were present in the same lungs, it was relatively unusual to find the two conditions near each other, which may suggest an antagonism between the cancerizing process and the tubercular process of progressive massive fibrosis (cf. the same thing in silicosis).

Graphite pneumoconiosis according to Parmeggianni (1950) is a relatively mild condition in spite of the 11% SiO_2 in Italian graphite (56% C, 11% SiO_2, 8.6% Al_2O_3, 3% Fe_2O_3), but the modified action of the silica does not appear to entail an increase in cancer.

6. Gas and Coke

Kennaway & Kennaway (1947) found that the certified deaths from cancer in gas-workers during 1921–38 exceeded the expected by 29–184%. Doll (1952) examined the mortality data of 840 pensioners over 60 years of age from a single large London gas-company, who died in the period 1939–48. The expected deaths from lung cancer were 10.4 calculated from data for England and Wales, and 13.8 from estimated London data, whereas 25 were found (P less than 0.001 and 0.01 respectively). This is very significant. Sixteen of the 25 men had probably worked in contact with tar, 23 having been employed in the gas-works for 30 years or more. Doll found only 4 fatal cases of skin cancer as against 2.4 expected from rates for England and Wales; these seem few when we note that Fisher (1953), at a tar distillery in South London, found that 60–100% of his men exposed to tar for 30 years or more showed varying degrees of skin changes.

The great improvement in working conditions may now have removed the twofold hazard of lung cancer found by Doll (1952) in pensioned gas-workers. In the National Coal Board's coking-plants Reid & Buck (1956) could find no evidence among men dying between 1949 and 1954 to indicate an increased hazard of lung cancer to all categories of workers. This may be related to the newer retort designs and the less emphasis on cracking and illuminant production in both coal-gas and coke processes.

7. Pitch, Tar, Soot and Mineral Oil

In some sense the problem of industrial skin cancers has been resolved, inasmuch as the clinical incidence, signs, symptoms, treatment and prognosis are understood and experimentally one or more carcinogens have been isolated from soot, pitch and tar. The active agents in mineral oil have not been identified. Fatal cases still occur. In 1955, 211 cases (18 fatal) were notified to the Chief Inspector of Factories: 48 due to mineral oils and 163 to pitch and tar. A classical fatal case of chimney-sweep's scrotal cancer on the left side (see Henry, 1946)[2] was reported in the same year. Goulden & Tipler (1949) obtained from domestic soot the fluorescence equivalent of 290 mg./kg. of 3:4-benzopyrene. The cutting-oil formulations used in machine-shops vary according to the speed of the tools. For low speeds mineral, vegetable or animal oil (with addition of phosphorus, sulphur or chlorine compounds) suffices; but, for high speeds, oil–water emulsions containing surface-active agents, rust inhibitors and disinfectants are used. Much oil is thrown off the machine and contaminates the workers. Cruickshank & Squire (1950) found that about one-third of 138 workers in three factories had hyperkeratoses or warts, increasing with length of service. Cruickshank & Gourevitch (1952) found, of 37 cancers of the hand and forearm (1941–50) and 34 cancers of the scrotum (1940–48) recorded in Birmingham, 30 derived from 88,859 meta-workers and 41 from the whole) remaining population of solme 271,000. Mastromatteo (1955 found six cases of squamous carcinoma and one of wart in a

[2] See also Henry, *Brit. med. Bull.* 1947, 4, 389.—ED.

machine-shop in Canada, and Gilman & Vesselinovitch (1955) confirmed this experimentally (cf. Blanding, King, Priestley & Rehner, 1951) on mouse skin with the soluble oil formulations which produced so much higher a response than that obtained by Woodhouse (1951, 1952) with mineral oil fractions that they cast suspicion on the additives in the formulations. Cruickshank & Squire (1950) found that rabbit skin was more responsive than mouse skin; and also Hieger & Woodhouse (1952), in very careful experiments, showed conclusively that rabbit skin is much more responsive to certain oil fractions and stated that ". . . it is unsatisfactory to exclude carcinogenicity on the basis of tests on mice only". Berenblum & Schoental (1947) had come to similar conclusions with certain constituents of coal-tar. But Woodhouse (1951, 1952) found 9:10-dimethyl-1:2-benzanthracene more potent on mice than on rabbits. The great need is for quantitative analysis of mineral oils, but this, as stated by Hieger and Woodhouse, is an immensely difficult matter.

8. Aromatic Amines

Goldblatt (1947, 1949) reviewed the problem of cases of occupational cancer of the bladder induced by aromatic amines and analysed 100 cases collected in two factories in the period 1934–47. Case and his co-workers (Case, 1953; Case, Hosker, McDonald & Pearson, 1954) made a statistical analysis of all death-certificates in England and Wales for 1921–50, in which tumour of the bladder was mentioned. From the data thus obtained the following results emerged:

Nominal roll of workers engaged in (i) manufacture, (ii) use or (iii) purification of one or more of aniline, 1-naphthylamine, 2-naphthylamine and benzidine . .	4,622
Expected number of death-certificates (cause: cancer of the bladder) in the three groups allowing for age and date of entry into the industry	3–5
Number of cases of bladder tumour in the three groups	at least 341
Number of these 341 cases which had contact with one or more of 1-naphthylamine, 2-naphthylamine and benzidine . .	298
Including aniline (4) and magenta (9) .	311
Number of these 311 who appeared in nominal roll, i.e., worked in the industry for more than six months . . .	262
Number of death-certificates (cause: cancer of the bladder) among the 262 . .	127

Thus, the over-all risk of dying from bladder cancer in the manufacture of synthetic dyestuffs was about 30 times that in the general population. It was also concluded that there was a definite hazard of bladder cancer in the manufacture of auramine (a diphenylmethane dyestuff) and of magenta (a triphenylmethane dyestuff). It may be recalled that the earliest cases of occupational bladder cancer described by Rehn (1895) were among workers in magenta manufacture. Case & Pearson (1954) found no evidence to suggest that the use or manufacture of aniline during 1910–52 has been a cause of bladder cancer.

From Case's analysis the number of fatal tumours already found among the manufacturing workers was 243 out of 2,466 during 1915–50, and the calculated forecast is that a further 243 fatal tumours will be found, even if no further exposure takes place.

Scott & Williams (1957) have presented a valuable guide to practice in industries making or using bladder carcinogens. The substitution of Tobias acid (by amination of sulphonated 2-naphthol) for the old method of first aminating the naphthol and then sulphonating the 2-naphthylamine, obviates the need for separation of the free amine, thus eliminating the cancer hazard, provided that conditions are such as to minimize the instability of the acid as well as the incomplete sulphonation of the naphthol.

Benzidine, an undoubted carcinogen in men and animals, is closely related to the important intermediates *o*-tolidine, dianisidine and dichlorobenzidine, but these compounds are not carcinogenic. 1-Naphthylamine, however made, contains 2-naphthylamine which probably constitutes its hazard. The *N*-alkyl and *N*-aryl derivatives of the naphthylamines are not carcinogenic. Sulphonation of the bases eliminates carcinogenicity. The making of naphthionic acid and its salts (1-naphthylamine-4-sulphonic acid) entails hazard because the amine is the starting material; the sulphonation is not complete, thus leaving free 1-naphthylamine and 2-naphthylamine in the wash-waters and in the tar residues.

Case & Hosker (1954) traced statistically that during 1936–1950 there appeared a marked increase in a large centre of the rubber industry and associated it with the use in that period of a condensation product of the naphthylamines with metaldehyde, which, however, contained 2–5% of unreacted bases. The material was at once withdrawn from manufacture and use; but the potential of bladder tumours in exposed workers will require many years to discharge.

Walpole, Williams & Roberts (1952, 1954) having shown that 4-aminodiphenyl was a potent bladder carcinogen in dogs, there followed the report of Melick, Escue, Naryka, Mezera & Wheeler (1955) that in an American factory where this compound was manufactured from 1935 till 1955, 11.1% of 171 workers developed bladder tumours, the induction time varying from 5 to 19 years and the exposure times from 1½ to 19 years. The rubber antioxidant made from this compound by condensation with an aliphatic ketone does not appear to be a bladder carcinogen when fed for long periods to dogs (personal communication from Dr E. R. Wheeler).

For the control of amino compounds in the clothing and urine of workers, analytical methods have been given by Butt & Strafford (1956) and Glassman & Meigs (1951). For the early diagnosis of bladder tumours Crabbe (1952) and Crabbe, Cresdee, Scott & Williams (1956) have described the application, in the field, of the Papanicolaou technique and the results of the examination of 1,800 workers. O 63 men examined both by the cytological technique and by cystoscopy, the cytological procedure failed to anticipate the later cystoscopic findings in only 6; but of 28 men later found to be cystoscopically negative, 10 were reported positive by cytology. The detection of abnormal cells in the urine is greatly facilitated by the concentration method of Rofe (1955, 1957).

Bonser, Clayson & Jull (1951)[3], using the method of Clayson (1950) for estimating 2-amino-1-naphthol conjugates, showed that the susceptibility of various species to the carcinogenic action of naphthylamine (*per os*) was directly

[3] See also Bonser, Clayson & Jull, p. 146 of this number of the Bulletin. —Ed.

related to the fraction of the dose thus eliminated and in the following order: rabbit:rat:mouse:dog as 1:2:4:5. Moreover, in the dog the urine–plasma concentration was about 200. This difference is evidently a real characteristic, for Henson, Somerville, Farquharson & Goldblatt (1954); Somerville, Henson, Cooke, Farquharson & Goldblatt (1956) and Somerville, Henson, Cooke and Goldblatt have injected 2-[8-^{14}C]naphthylamine intraperitoneally in dog, rabbit, guinea-pig, mouse and rat and found 87%, 86%, 84%, 80% and 65% respectively of the dose excreted in the urine but at different rates (paper in preparation).

Bonser, Clayson, Jull & Pyrah (1956) showed that all their dogs that had been fed 2-naphthylamine (purified or very highly purified[4] by Case's method of gradient sublimation) for two years or more (max. cumulative dose, 310 g.) developed bladder tumours. Arachis oil solution of 2-naphthylamine allowed to stand for four weeks and injected subcutaneously induced local sarcomata and hepatomata; freshly prepared solutions were much less effective in this respect. Using Jull's technique (1951) for implantation of paraffin pellets containing the base into the bladder of mice, Bonser, Bradshaw, Clayson & Jull (1956) induced carcinoma, benign tumours and squamous metaplasia, but to a much less extent than with the metabolite 2-amino-1-naphthol. Similar results were obtained with 4-aminodiphenyl. The authors conclude that both these bases possess slight carcinogenic activity; but see also Bonser, Crabbe, Jull & Pyrah (1954). With 1-amino-2-naphthol, a very high yield of tumours was obtained; similarly with 3-hydroxy-4-aminodiphenyl and its 4′-nitro derivative. These findings strongly sustain the *ortho*-hydroxyamine theory. However, some nitrogenous compounds injected directly into the bladder may act as carcinogens, as was shown by Bonser, Clayson & Jull (1954) with 3 : 4-5 : 6-dibenzocarbazole (0.25% weekly for 12 months—transitional cell papillomatosis 2½ years later), and was first observed by skin-painting by Boyland & Brues (1937) and Boyland & Mawson (1938); sarcomata were produced by subcutaneous injection, and sarcomata and cholangiomata by intraperitoneal injection.

[4] Impurities commonly found in 2-naphthylamine include 1-naphthylamine, pyrene, 3 : 4-5 : 6-dibenzocarbazole, 2 : 2-dinaphthylamine, 1 : 2-5 : 6-dibenzophenazine (Case & Pearson, 1952).

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AROMATIC AMINES AS CARCINOGENS IN INDUSTRY

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1 Dyestuffs industry
2 Other industries
3 Examination of new aromatic amines
a Aminodiphenyl derivatives
b Aminostilbene derivatives
4 Conclusions
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1. Dyestuffs Industry

The history of cancer as an occupational disease is closely linked with that of the manufacture of synthetic dyestuffs from coal-tar. This industry dates from Perkin's exploitation of his discovery of mauve, accidentally made, in 1856, when he was trying to synthesize quinine (Perkin, 1856). The manufacture of triphenylmethane dyes, which rapidly developed from this beginning, required for the first time the production on a large scale of aromatic amines—aniline and the toluidines and xylidines—from coal-tar. For a long time these amines were used for the manufacture of dyes as crude mixtures of variable composition, characterized only by the ranges of temperature over which they distilled. Their acute toxic effects on man were quickly appreciated as a result of unfortunate experiences, but chronic toxic effects were not expected.

Nearly 40 years later, however, Rehn (1895) drew attention to the fact that four men whom he was treating for tumours of the urinary bladder all worked in the same dyestuffs factory, and further inquiry revealed that they were all employed in making the same dye, fuchsin (magenta). Rehn's suggestion that the tumours in these men were due to their environment was received with scepticism which increased as a further 60 years passed and no further tumours were attributed to the manufacture of this dyestuff in any country. But in 1954 Case & Pearson published statistical evidence showing that there had been a significant excess of bladder tumours in Great Britain, during the previous 35 years, amongst men who made magenta. The facts established by these observations, 60 years apart, have not yet been adequately explained, despite an abundance of theories. The suspicion has been entertained at one time or another that aniline can cause cancer of the bladder, which for many years was known in the industry as " aniline cancer ", and Hergt (personal communication) believed that some of the early cases in Germany could be attributed to toluidines; but there is no satisfactory evidence that any of the simpler aromatic amines already mentioned are, in fact, carcinogenic. Walpole, Williams & Roberts (1952) suggested that the early tumours in the German industry were due to contamination of these amines by naphthylamines and xenylamines, but such an explanation is scarcely applicable to tumours occurring in British workmen as late as 1920–53. It has also been suggested that Rehn's cases were exposed to other aromatic amines such as 2-naphthylamine and benzidine, which are now known to be bladder carcinogens in man, but in the British factories in question these amines were not made or used. The possibility that magenta itself, a triphenylmethane dyestuff, is carcinogenic, has not been neglected. Yoshida, Schimauchi & Kin (1941) claim to have obtained papilloma of the bladder in rats following intravesical implantation of the dyestuff in collodion, but Willheim & Ivy (1953) saw no tumours of any kind in rats to which it was given in the diet in high concentration, and in the experiments of Bonser, Clayson & Jull (1956) no significant yield of tumours resulted in mice from repeated oral dosing.

The sulphonated triphenylmethane dye, Light Green SF Yellowish (Schiller, 1937; Harris, 1947), produces sarcoma at the site of its repeated subcutaneous injection in aqueous solution in rats. No distant tumours are produced, however, and when given by mouth the dye appears inactive (Allmark, 1956). There is no evidence whatever that its manufacture is associated with any hazard to the workers involved.

Two cases of bladder tumour were attributed by Müller in 1933 to the manufacture of the dyestuff auramine, a derivative

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of diphenylmethane. This manufacture was incriminated on statistical grounds by Case & Pearson in 1954. They described eight more cases, in Great Britain, attributable to this cause. Bonser *et al.* (1956) produced a few hepatomata in mice with a commercial sample of the dyestuff given in the diet, but all the chemicals involved in its manufacture have not been fully investigated and a conclusive explanation for the bladder tumours in workmen making this dyestuff has not yet been reached.

The discovery by Griess of diazotization in 1858 and of the coupling of diazonium salts in 1864 led to the development of a new class of coal-tar dyestuffs, the azo dyes, for which new aromatic bases, including the naphthylamines and benzidine, were required as intermediates. Occupational cancer of the bladder was first attributed to naphthylamine at the turn of the century (Leichtenstern, 1898). 2-Naphthylamine was accepted as a cause, on epidemiological evidence, shortly after the First World War (International Labour Office, 1921) and 16 years before bladder tumours were induced with it in dogs (Hueper & Wolfe, 1937; Hueper, Wiley & Wolfe, 1938). Although no experimental evidence is available to incriminate 1-naphthylamine, Case, Hosker, McDonald & Pearson (1954) presented evidence of a significant excess of bladder tumours amongst both users and makers of the commercial product, which contains 4% of 2-naphthylamine, 23 years after cases were first attributed to it by Wignall (1929).

Benzidine, which has been under suspicion since the 1920's, was not accepted as a cause by the Americans Gehrmann, Foulger & Fleming as late as 1949, despite the attribution to it of numerous cases by workers in different countries. For many years, Maguigan in the USA maintained that benzidine was a cause of bladder cancer in industrial workers, and in 1950 Spitz, Maguigan & Dobriner demonstrated its carcinogenicity in rats and dogs. Case *et al.* (1954), in their survey of the British chemical industry already referred to, obtained statistical evidence that a high incidence of bladder tumours had developed in men exposed to benzidine, and so replaced suspicion by statistical proof.

Suspicion that azo dyes themselves might be carcinogenic was aroused by early work by Fischer (1906) on scarlet red. Intensive study of the subject was initiated by Japanese workers in the early 1930's and taken up later by others, including Orr, Kirby and Peacock in Great Britain, and Shear and more recently the Millers in the USA. *o*-Aminoazotoluene was found to be a potent liver carcinogen in rats and mice, and to produce bladder tumours in rats. 4-Dimethylaminoazobenzene (butter yellow) was found to be even more active in inducing liver tumours. Numerous related azo compounds have since been examined and many of these produce neoplasms, particularly of the liver, in rats and mice. Work in this connexion has often been reviewed (Miller & Miller, 1953, 1955; see also Miller, Miller & Finger, 1957), and will not be discussed further here. Bladder tumours have been produced in dogs with *o*-aminoazotoluene and butter yellow (Nelson & Woodard, 1953) but there is as yet no published evidence that any azo compound has caused cancer in man.

2. Other Industries

Wilson, De Eds & Cox in 1941 made a toxicological study of 2-acetamidofluorene, which was of interest on account of its insecticidal properties. They found that on continued oral administration to rats it produced tumours of several organs, notably the bladder, liver and lungs. As a result the compound has never been manufactured on a scale sufficient for its carcinogenic action to become an industrial hazard. Its versatility as a carcinogen was confirmed by later work, reviewed by Bielschowsky in 1947. More recently Morris & Eyestone (1953) have obtained bladder tumours with it in dogs. Its action and that of numerous related substances have been widely studied, especially during recent years by the Millers and their associates in Madison, Wisconsin, and by Morris and his co-workers in Bethesda, Maryland (Miller, Sandin, Miller & Rusch, 1955; Miller & Miller, 1955; Morris, 1955).

Interest in 4-aminodiphenyl (xenylamine) as a potential carcinogen was first stimulated by learning from the literature that it had been isolated by Hofmann in 1862 from the crude mixture of bases derived from coal-tar which was an intermediate stage in the manufacture of triphenylmethane dyes (Walpole *et al.* 1952), and also by the demonstration of carcinogenic activity in the related substances *NN*-dimethyl-4-aminodiphenyl (Miller, Miller, Sandin & Brown, 1949) and benzidine (Spitz *et al.* 1950). As 4-aminodiphenyl produced a variety of tumours in rats (1952) and bladder tumours in dogs (1954), Walpole, Williams & Roberts forecast that bladder tumours would develop in men exposed to it. Melick, Escue, Naryka, Mezera & Wheeler (1955) found bladder tumours in 19 men engaged in its manufacture in the USA since 1938. Thus it had taken 17 years for this amine to manifest its carcinogenic potential and during that time 171 men had been exposed to risk. It will be many years before the full extent of the damage caused by this manufacture, now abandoned, will be known. In Great Britain manufacture of 4-aminodiphenyl was never started, because of the experimental evidence quoted.

One of the uses of 4-aminodiphenyl in the USA was in the manufacture of an antioxidant for rubber (Case, 1954). A similar condensate, prepared from naphthylamine and an aldehyde, was marketed in Great Britain as a rubber antioxidant as early as 1927. Its manufacture was abandoned in 1949 when the danger from free naphthylamines in the final product became apparent. Case & Hosker (1954) demonstrated an excess of cases of bladder tumours in rubber workers in a county borough where this naphthylamine aldehyde condensate had been used for many years. The fate of American rubber workers exposed from 1938 to 1955 to the xenylamine condensate is no doubt being followed with concern.

While considering rubber workers it is well to remember that the use of benzidine as a hardening agent is still advocated in text-books of rubber technology. Such a use must be regarded as hazardous. Both benzidine and 1-naphthylamine have also been extensively used in textile printing. Although no tumours have been attributed to their use in this industry it would seem advisable to handle them there with precautions similar to those in the chemical industry.[1] Benzidine has been used for many years in the manufacture of " security paper " to produce a stain in the presence of bleach and thus to expose attempts to delete and alter writing (Simons, 1951). It is expected that this use of the compound will produce its quota of tumours. The suggested use for this purpose of other carcinogens, including naphthylamines and aminodiphenyls (Simons, 1937) and 4 : 4′-diaminostilbene (Smith, 1937),

[1] See also Goldblatt, p. 136 of this number of the Bulletin.—Ed.

which produces a high incidence of liver tumours in rats (Walpole & Williams, unpublished), must also be regarded as hazardous.

The human exposure involved in using benzidine in clinical and forensic laboratories as a test agent for occult blood is probably insignificant, but its use for spraying chromatograms in biochemical laboratories must be regarded as potentially hazardous.

Many other carcinogenic aromatic amines already exist, and it is inevitable that technologists will look for new amines to produce improved products and unique technical effects.

3. Examination of New Aromatic Amines

With the object of learning more about the effects of ring substitution on the carcinogenic properties of amines known to be active in this respect, we have examined a number of substituted aminodiphenyls, diaminodiphenyls, aminostilbenes and diaminostilbenes. The results will be published in detail elsewhere, and we give only a summary of the more significant of the findings.

a. Aminodiphenyl Derivatives

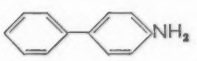
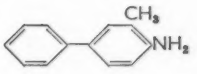
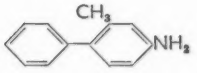
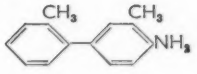
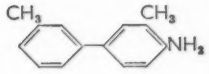
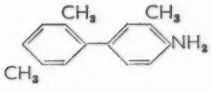
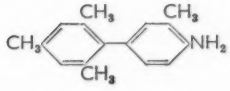
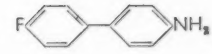
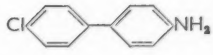
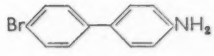
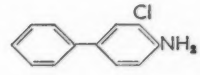
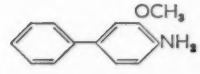
Table I relates to derivatives of the 4-aminodiphenyl series. In the first instance these compounds were given by repeated subcutaneous injection in arachis oil, at doses near the maximum tolerated, to groups of 24 male albino Wistar rats. For each are shown in Table I the total dose in g./kg. and the main sites of tumour incidence in descending order of frequency. Sarcomata at the injection site, which developed late in the experiments with several of these substances, are probably to be attributed to the oily vehicle and are not included.

Consideration of the detailed results of these experiments in rats leads to the conclusion that the introduction of a methyl group into the 3-position in 4-aminodiphenyl, with or without methyl groups in other positions, enhances carcinogenic efficacy, especially for the intestine. Methyl substitution in the 2-position, on the other hand, results in a compound which produces liver tumours only, and these in relatively low yield, while 4′-methyl-4-aminodiphenyl, in very large doses, causes tumours of the acoustic sebaceous gland, intestine and liver in descending order of frequency.

The results obtained with 4′-fluoro-4-aminodiphenyl, which produces tumours of the liver and kidney in high yield (Hendry, Matthews, Walpole & Williams, 1955)[2], and with its chloro- and bromo-analogues, are in line with the observations of other workers who have fed to rats derivatives of 2-acetamidofluorene halogenated in the 7-position. Thus 7-fluoro-2-acetamidofluorene is more active as a carcinogen than acetamidofluorene itself (Miller *et al.* 1955), while the corresponding chloro-compound is less active and the iodo-derivative appears inactive at any site (Morris, 1955). Even in the butter-yellow series of liver carcinogens, substitution of fluorine, chlorine and bromine in the 4′-position has similar over-all effects upon carcinogenic potency (Miller, Miller & Finger, 1953; Miller, Sapp & Miller, 1949; Kuhn & Quadbeck, 1949).

The activity of 3-methoxy-4-aminodiphenyl in producing bladder tumours recalls a similar result obtained by Hackmann (1956) on feeding 2-amino-3-methoxydiphenylene oxide to rats.

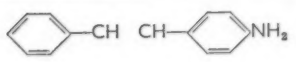
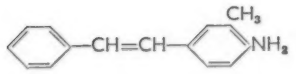
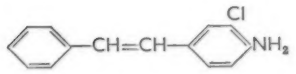
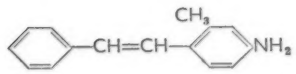
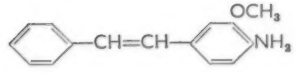
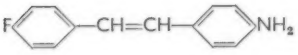
TABLE I. TUMOURS PRODUCED IN RATS BY THE REPEATED SUBCUTANEOUS INJECTION OF 4-AMINODIPHENYL AND SOME DERIVATIVES THEREOF

Compound	Total dose (g./kg.)	Organs in which tumours arose in significant yield
	5.0	Intestines, liver
	1.2	Intestines, acoustic sebaceous gland
	2.4	Liver
	10.1	Acoustic sebaceous gland, intestine, liver
	2.8	Intestines, acoustic sebaceous gland
	2.4	Intestines, acoustic sebaceous gland
	1.4	Intestines, liver
	1.4	Intestines, liver
	2.0	Kidney, liver, intestines
	3.3	No significant yield at 700 days
	4.1	No significant yield at 800 days
	7.0	Intestines
	4.4	Bladder

The derivatives of 2-aminodiphenylene oxide (aminodibenzofuran), and those of 2-aminofluorene, constitute two series of compounds which may be regarded as derived from the corresponding substitution products of 4-aminodiphenyl by the

[2] See also Matthews & Walpole (1958) *Brit. J. Cancer*, 12 (In press)

TABLE II. TUMOURS PRODUCED IN RATS BY THE REPEATED SUBCUTANEOUS INJECTION OF 4-AMINOSTILBENE AND SOME DERIVATIVES THEREOF

Compound	Total dose (g./kg.)	Organs in which tumours arose in significant yield
	0.2	Acoustic sebaceous gland (variable yield of local sarcomata)
	0.2	Local sarcomata only (less than 190 days)
	0.2	Local sarcomata only (less than 200 days)
	0.24	Local sarcomata only (less than 250 days)
	0.25	Local sarcomata only (302–625 days)
	0.2	Acoustic sebaceous gland, liver, intestines

insertion of an —O— or —CH_2— bridge in the 2:2′-position. Carcinogenic activity in these series, and in others with different bridging atoms or groups, has been investigated and kept under review, on a comparative basis, by the Millers and their associates (see their publications from 1955 onwards).

b. Aminostilbene Derivatives

In view of their possible future use in industry, we have carried out similar tests with analogous substitution products in the 4-aminostilbene series. Haddow, Harris, Kon & Roe (1948) obtained local sarcomata at the site of subcutaneous injection, carcinomata of the "acoustic duct" and mammary adenomata in rats with 4-aminostilbene, 4-*NN*-dimethylaminostilbene, 4-*NN*-dimethylamino-2′-methylstilbene and 1-(4′-*NN*-dimethylaminophenyl)-2-(1′-naphthyl)-ethylene. In our experiments 4-aminostilbene and its substitution products were given to male rats by repeated subcutaneous injection in arachis oil (daily or twice weekly) up to a total in all cases of about 0.2 g./kg. The results shown in Table II may be summarized as follows. 4-Aminostilbene itself produced a high yield of carcinomata of the acoustic sebaceous gland (after 175–642 days), with local sarcomata in a yield which appears to depend in some way on the precise schedule of dosing. With 3-methyl-, 3-chloro- and 2-methyl-4-aminostilbene, local sarcomata only were observed. These tumours appeared in almost every animal treated and all arose between 150 and 250 days after injection. These tumours must be attributed to the specific action of the respective compound and not to the oily vehicle used for its injection. In one feeding experiment with 3-methyl-4-aminostilbene (average total dose 0.35 g./kg.), two of four animals surviving for 190 days or more developed tumours of the acoustic sebaceous gland. The injection of 3-methoxy-4-aminostilbene produced local sarcomata in 18 of 24 animals, but these tumours appeared much later (302–625 days). 4-Amino-4′-fluorostilbene gave the same high yield of tumours of the acoustic sebaceous gland as 4-aminostilbene itself and produced tumours of the liver and intestine in significant yield but no local sarcomata.

Fewer compounds in the 4 : 4′-diaminostilbene series were tested. We have shown that the parent diamine, in a total dose of 0.2 g./kg., produces tumours of the liver, with some late local sarcomata. On the other hand 4:4′-diamino-3:3′-dichlorostilbene, in comparable dose, produced only local sarcomata. These occurred in all but one of 24 animals in 200–400 days. 4:4′-Diamino-2:2′-dichlorostilbene was less toxic and, given in a total dose of 1.4 g./kg., yielded liver tumours in 18 of 24 animals, a few tumours of the intestine and local sarcomata.

The interpretation of these results, and in particular the explanation of the differences in the site of action of these related chemicals, must await further experiment. In the meantime they show that carcinogenic activity in some form is a property common to many derivatives of 4-aminodiphenyl and 4′ : 4′-diaminodiphenyl and of 4-aminostilbene and 4 : 4′-diaminostilbene.

4. Conclusions

The history of occupational cancer of the bladder shows that many years may elapse before the danger from an industrial carcinogen is recognized or even suspected. Sixty years of scepticism passed before Rehn's original indictment of fuchsin manufacture in Germany was confirmed in Great Britain; 23 years elapsed between the attribution of the first case to naphthylamine and the general recognition of the hazard from 2-naphthylamine; for over 30 years benzidine was under suspicion before it was universally accepted as a bladder carcinogen in man; 25 years passed before bladder tumours were discovered in rubber workers exposed to naphthylamine-aldehyde condensates in Great Britain and, as a more recent example, 4-aminodiphenyl had been manufactured in the USA for over 17 years before the danger involved was pointed out.

Every year that inadequate precautionary measures are taken, the final yield of bladder tumours increases. The chemical industry has taken active measures to combat this danger. The manufacture of 2-naphthylamine has been abandoned in Germany, Switzerland and Great Britain, and rubber antioxidants from 1- and 2-naphthylamine are no longer made in Britain. The publication by the Association of British Chemical Manufacturers of a code of practice for the handling of known bladder carcinogens (Scott & Williams, 1957) has laid down a standard to be aimed at in achieving safe working conditions. If all these measures are successful, the future yield of tumours due to known carcinogens in the dyestuffs industry should fall rapidly in the next 20 years, but the casual use of these dangerous substances in other industries must be a matter for concern.

We present the summarized results of our tests of amines and diamines as a guide to chemists and technologists who may contemplate using them in the future. In this way we

hope to anticipate some of the dangers from compounds of this nature and thus prevent a recurrence of the unfortunate experiences of the past.

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SOME ASPECTS OF THE EXPERIMENTAL INDUCTION OF TUMOURS OF THE BLADDER

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1 Tumours in man
2 Systemic application of carcinogens and the induction of bladder cancer
3 Local applications of carcinogens to the bladder
4 Mechanism of formation of aromatic amine tumours
5 Spontaneous bladder tumours
6 Conclusions and future prospects
References

1. Tumours in Man

The necessity for the study of bladder cancer is emphasized by the realization that workmen making, using or purifying certain products of the chemical industry are liable to a higher incidence of cancer of the bladder than the general population. Goldblatt (1947)[1] has reviewed our knowledge of the human disease; the position has been greatly clarified by the results of a field survey undertaken by Case and his colleagues (Case, Hosker, McDonald & Pearson, 1954; Case & Pearson, 1954). There can now be no doubt that the manufacture or handling of 2-naphthylamine, 1-naphthylamine and benzidine, but not aniline, is responsible for a large proportion of the industrial tumours. Whether 1-naphthylamine itself, or the 5–10% of 2-naphthylamine which the technical chemical always contains, is responsible for the observed tumours with this compound is uncertain. Case & Pearson (1954) also showed that the manufacture but not the handling of auramine and magenta is hazardous.

It would be surprising if the five chemicals listed above proved to be the only substances capable of inducing bladder cancer in man in our increasingly technological environment. That others do exist has been shown dramatically by Melick, Escue, Naryka, Mezera & Wheeler (1955), who found that workmen manufacturing 4-aminodiphenyl for periods of 1¼–19 years developed bladder tumours 5–19 years after their initial exposure.

Other industrial processes in which bladder tumours have at one time or another been observed are listed by Hueper (1942). These include exposures to coal-tar and pitch, etc., but the suggested environmental aetiology of such bladder tumours is supported by clinical and statistical evidence only and must therefore remain in doubt. Bilharzial infection, common in the lower Nile delta, is believed to be associated with the high incidence of bladder cancer occurring in that area. These possible causative agents will not be considered in this article as little work on their mode of action has been published.

The known causes of bladder cancer in man are listed in Table I. The experimental investigator wants to determine the answer to the following questions: What is the nature of the mechanisms by which the industrial aromatic amines induce bladder tumours? What substances not at present in common use are likely to be hazardous if used on a large scale without adequate precautions? Does the knowledge of the mechanism of the formation of the industrial tumours help us to understand and possibly to eradicate the "spontaneous" disease in the general population?

2. Systemic Application of Carcinogens and the Induction of Bladder Cancer

In the experimental reproduction of the human disease, the investigator can either administer the carcinogen by systemic routes such as feeding, injection, inhalation, etc., or can apply it locally to the bladder epithelium. Various successful attempts to induce bladder cancer by the systemic application of carcinogens (Table II) show that bladder cancer can be induced in many species, but the ease with which tumours arise with a given agent depends to a great extent on the species and strain of the test animal. It is also clear that tumours occur at sites other than the bladder and that certain aromatic amines and azo compounds are the common effective carcinogens. These points will be discussed briefly.

The difference in species susceptibility to a given agent is well illustrated by 2-naphthylamine. When dogs are adequately dosed with the chemical, almost all develop bladder tumours in 2–5 years (Hueper, Wiley & Wolfe, 1938; Bonser, 1943). In contrast to this, papillomata of the bladder developed in rats in 60–90 weeks and early neoplastic changes in the bladder epithelium of rabbits were seen only after 5 years (Bonser, Clayson, Jull & Pyrah, 1952). The mouse, on the other hand, did not give bladder tumours but hepatomata in 70–90 weeks (Bonser *et al.* 1952; Bonser, Clayson, Jull & Pyrah, 1956). The difference in strain susceptibility within a species is well illustrated in the case of 2-acetamidofluorene administered to the mouse and rat. Armstrong & Bonser (1947) showed that the incidence of bladder tumours with this chemical in CBA, IF, RIII, White label and Strong A mice was 78, 70, 48, 22 and 22% respectively. Wilson, De Eds & Cox (1941) showed that 2-acetamidofluorene induced a fair yield of bladder tumours in their Slonaker rats, whereas Bielschowsky (1944) found no bladder tumours in his Wistar rats but noted instead tumours of the intestine. Similarly, Walpole, Williams & Roberts (1955) showed that Slonaker rats dosed with 3 : 2′-dimethyl-4-aminodiphenyl gave a high yield of bladder tumours, whereas their Wistar strain did not.

Those agents which cause bladder cancer are able in many cases to induce tumours at other sites. 2-Acetamidofluorene is probably the most ubiquitous systemic carcinogen known (Table II). On the other hand 2-naphthylamine is much more limited in its scope, its action being confined to the bladder and liver. Other amines will probably be found to occupy intermediate positions when they have been tested fully.

[1] See also papers by Goldblatt (p. 136) and by Walpole & Williams (p. 141) in this number of the Bulletin.—Ed.

TABLE I. INCIDENCE OF BLADDER TUMOURS IN MEN EXPOSED TO DYESTUFFS OR THEIR INTERMEDIATES

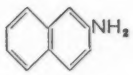
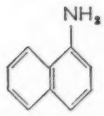
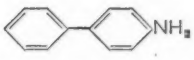
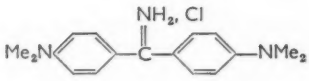
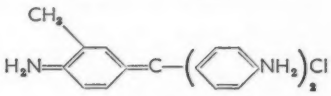
Compound	Formula	Incidence of bladder cancer		Reference
		Expected in general population	Found in dyestuffs operatives	
2-Naphthylamine		0.30	59	Case et *al.* (1954)
1-Naphthylamine		0.70	28	Case et *al.* (1954)
Benzidine		0.72	38	Case et *al.* (1954)
4-Aminodiphenyl		Not known	19 (Population at risk 171; compound first manufactured 1935)	Melick et *al.* (1955)
Auramine		0.45	8	Case & Pearson (1954)
Magenta		0.13	6	Case & Pearson (1954)

TABLE II. INDUCTION OF BLADDER TUMOURS BY THE SYSTEMIC APPLICATION OF CARCINOGENS IN VARIOUS SPECIES

Compound	Species	Strain	Bladder tumours	Latent period (years)	Other tumours	Reference
2-Naphthylamine	Dog	—	PC	2–5	—	Hueper (1938)
	Rat	Albino	P	1–2	Liver	Bonser et *al.* (1952); Boyland et *al.* (1954)
	Rabbit	—	E	5	—	Bonser et *al.* (1952)
2-Aminofluorene (2-Acetamidofluorene)	Dog	Beagle	PC	6–8	Liver	Morris & Eyestone (1953)
	Rat	Various	PC	1–1½	Liver, intestine, breast, ear duct, etc.	Dunning, Curtis & Madsen (1947)
	Mouse	See text	PC	1–1½	Liver, breast, etc.	Armstrong & Bonser (1947)
	Rabbit	—	PC	4–5	Ureter	Bonser & Green (1950)
Benzidine	Dog	—	C	7–10	—	Walpole, Williams & Roberts (1954)
1-Naphthylamine	Dog	—	EP	6–8¾	—	Bonser, unpublished results
4-Aminodiphenyl	Dog	Beagle	PC	2¾	—	Walpole et *al.* (1954)
3 : 2'-Dimethyl-4-amino-diphenyl	Rat	Slonaker	PC	¾	Intestine, ear duct, salivary glands	Walpole et *al.* (1955)
o-Aminoazotoluene	Dog	—	PC	2½–3½	Gall-bladder, liver	Nelson & Woodard (1953)
	Rat	—	PC	—	Liver	Hartwell (1951)
p-Dimethylaminoazobenzene	Dog	—	PC	3–4	—	Nelson & Woodard (1953)
3-Methoxy-2-aminodiphenyl-ene oxide	Rat	—	C	—	Ureter, kidney	Hackmann (1956)

E: Early neoplastic changes **P: Papillomata** **C: Carcinomata**

The differences in species susceptibility and site of action of the bladder carcinogens makes the choice of test species for a new chemical very difficult. Mice or rats have the great advantage that they may be kept economically in adequate numbers for the statistical appraisal of the biological test. On the other hand most amine tumours arise late in the life of the mouse (60–80 weeks) and some amines (e.g. 2-naphthylamine) have but little effect on the rat. Rabbits and, to a greater extent, dogs are expensive to keep and only a small number can normally be devoted to the test of any one substance. This means that, unless the compound induces tumours in a high proportion of animals, considerable doubt will arise as to whether the few tumours obtained may be attributed to the chemical or are spontaneous. Also it is generally true that, the longer the normal life-span of a species, the longer is the latent period of the induced tumours.

The majority of the bladder tumours listed in Table II were induced by aromatic amines or azo compounds. It has been suggested that impurities in the aromatic amines such as compounds akin to the polycyclic hydrocarbons are the cause of the tumours. Thus Case & Pearson (1952) induced sarcomata in mice by subcutaneous injection of an oily solution of a concentrate of the impurities in technical 2-naphthylamine. This suggestion as to the true causative agent is contradicted by the demonstration (Bonser, Clayson, Jull & Pyrah, 1956) that 2-naphthylamine purified by one of the most rigorous methods available (gradient sublimation) is still as carcinogenic to the dog and mouse as the commercial chemical.

The species difference in response to the amine has led many workers to suggest that it is not the applied amines themselves that are carcinogenic but products of their metabolism in the animal body. Clayson (1952, 1953) noted, in reviews of the literature, that all the amines which were at that time known to be carcinogenic in any site had the *para* position to the amino group blocked by a large substituent. He suggested that: (i) compounds which contain an amino and a hydroxy group *ortho* to each other in a system containing two or more aromatic rings may be carcinogenic either in their own right or as a result of their further reaction; (ii) the reason why some aromatic amines induce tumours whereas others do not is that the former are more readily converted to *o*-hydroxyamines (*o*-aminophenols) than the latter. The systemic application of carcinogens is, by itself, insufficient for the testing of these ideas.

3. Local Applications of Carcinogens to the Bladder

In order to provide the optimum conditions for the testing of a chemical as a potential carcinogen, it is necessary to maintain as high a concentration as is consistent with the protracted survival of the test animal. This criterion limits the suitable methods for the testing of potential carcinogens directly on the bladder epithelium to the following: repeated intra-urethral injection in solution or suspension; the surgical implantation of the substance as a pellet either directly into the lumen of the bladder or into the submucosal layers; or the subcutaneous grafting of transplanted bladder epithelium wrapped round the test substance, where if the tissues live the changes may proceed to malignancy. The latter method was employed by Hughes (1949) who, using methylcholanthrene as the test substance, obtained 14 carcinomata of the bladder epithelium in 120 mice. This is a low yield of tumours with so potent a carcinogen.

Repeated intra-urethral injection of solutions into the lumen of the bladder is tedious and because of technical difficulties has been limited to the larger animals. Yamazaki & Sato (1937), cited by Hueper (1942), injected watery solutions of aniline into the bladder of rabbits in this way. Their conclusions were questioned by Bonser (1947), as tumours arose after 13 days. Bonser, Crabbe, Jull & Pyrah (1954) obtained bladder tumours in one dog by the weekly injection for 52 weeks of an oily solution of 3:4-5:6-dibenzocarbazole. The amount of labour demanded by this technique, and the expense involved in keeping dogs, militate strongly against the extended use of the method.

The need for a routine method for the testing of *o*-hydroxyamines and similar compounds led Jull (1951) to develop the technique of implanting paraffin wax pellets containing suspected substances into the lumen of the mouse bladder. This method has had several years of intensive trial in Leeds and is now finding acceptance elsewhere. Its theoretical advantages might be that the chemical diffuses out of the pellet, that the chances of metabolism of the chemical by the liver are minimized, that the bladder is able to function under approximately physiological conditions and that the chemical is being constantly replenished in the urine and so oxidation should be kept to a minimum. On the other hand the method has certain limitations, as the chemical and wax may interact in unknown ways while remaining in the bladder, and this change is likely to be enhanced by the solution of the test chemical in molten wax; also the effect of the presence of a foreign body on bladder carcinogenesis is not known, so that, even if the pellet is incapable of inducing bladder tumours per se, it may decisively influence the induction of tumours by chemicals. Similar difficulties are present in those conventional methods of testing carcinogens where solvents are used. The original implantation method was modified by Boyland & Watson (1956), who simplified the surgical technique and introduced compressed chemical and cholesterol mixtures instead of wax for the pellets. The Leeds workers are now trying crushed paraffin wax as the vehicle. Chalmers & Pullinger (1956) have shown that the ease of diffusion of certain steroids from paraffin pellets varies considerably and that it will be necessary to consider this factor in the choice of vehicle and in the evaluation of results.

Bonser & Jull (1956) discussed the histological changes which follow the implantation of pellets into the mouse bladder. In a statistical analysis of 467 mice implanted with pellets of paraffin wax either alone, or with a chemical, they found that, whereas concretion was significantly related to squamous metaplasia, and squamous metaplasia to carcinoma, concretion and carcinoma were not significantly related. They were unable to assess the relation between papilloma and carcinoma.

An attempt to use the method in rats was unfruitful, as the control animals developed lesions which made the interpretation impracticable (Bonser, Clayson, Jull & Pyrah, 1953). On the other hand Rudali, Chalvet & Winternitz (1955) implanted rats with pellets containing various phenazine derivatives, and found that 10 of 14 rats implanted with 3:4-5:6-dibenzophenazine had tumours, as against 0 of 5 implanted with wax alone. In all groups animals were still living, so that the final results will be awaited with interest.

An occasional tumour occurs in mice implanted with the vehicle alone. Bonser, Bradshaw, Clayson & Jull (1956) found that 2 of 56 mice implanted with paraffin wax developed carcinomata and 5 of 55 implanted with cholesterol (unpublished

observation). Allen *et al.* (1957) obtained 1 carcinoma in 24 mice implanted with cholesterol. This induction of tumours with the vehicle alone means that relatively large groups of animals must be used. Bonser, Bradshaw, Clayson & Jull (1956) suggested that groups of not less than 30 mice surviving for 25 weeks or more after implantation are required for the testing of potent carcinogens, and greater numbers for less potent chemicals, if significant results are to be obtained.

In Table III the results obtained by bladder implantation are summarized. Compounds which have been tested and found to be without activity in groups of less than 20 mice are omitted, as it is felt that results based on insufficient evidence may be misleading. The majority of the *o*-hydroxyamines tested are active carcinogens. Two azo compounds, both of which have been employed as food dyes, were found to possess extremely potent local activity. As a class the aromatic amines themselves show some activity. 2-Naphthylamine and 4-aminodiphenyl implanted in wax, as well as 2-aminofluorene in cholesterol, have definite though weak activity. 2-Anthramine in wax shows greater potency but the other amine derivatives tested show little or no activity.

4. Mechanism of Formation of Aromatic Amine Tumours

The hypothesis advanced by Clayson (1953) must now be rigorously reviewed in the light of the evidence which has accumulated since it was put forward. It was assumed that the active agent in induced bladder cancer is borne by the urine rather than the blood. McDonald & Lund (1954) demonstrated the urogenous origin of bladder tumours by preparing bladder pouches in dogs and then feeding 2-naphthylamine. Tumours were induced in the bladders themselves but not in the pouches unless these were defective and urine leaked in. In a similar experiment Scott & Boyd (1953) transplanted the ureters of dogs into a sigmoid loop of intestine and fed 2-naphthylamine to the dogs. They obtained neither bladder nor intestinal tumours, but where the ureters were constricted as a result of the operation ureteric tumours developed. This experiment suggests that stasis of the urine plays a part in the induction of 2-naphthylamine tumours.

The results of testing those aromatic amines which have no large substituent in the *para* position, and which would thus seem less likely to give rise to *o*-hydroxyamines, suggest that any activity possessed by these compounds is of a low order (Table IV). Some experiments by Bonser (unpublished observation) using aniline and 1-naphthylamine have a direct bearing on this point as well as on the industrial disease. When aniline (0.5 g. three times per week) was fed to three dogs for 6 years one hepatoma and one papilloma of the bladder occurred after 10 and 10½ years. The small numbers involved, the long induction period and the paucity of knowledge of the spontaneous tumour rate in dogs of that age, do not permit any definite conclusion to be drawn. Similarly with 1-naphthylamine (AnalaR), early neoplastic changes of the bladder epithelium were found in one dog after 6½ years, and a papilloma of the bladder after 8¾ years. AnalaR 1-naphthylamine contains some 3–5% of 2-naphthylamine, so that the dogs would have received about 25 g. of that potent carcinogen during the period of treatment, which might well be responsible for the observed tumours.

The suggestion that the *o*-hydroxyamines would prove to be carcinogenic (Clayson, 1953) has received support (Table III),

TABLE III. THE RESULTS OF IMPLANTATION OF CHEMICALS IN THE MOUSE BLADDER

Compound	Base	Carcinomata	Survivors	Activity
None[a]	P	2	56	—
None[b]	C	5	55	—
None[c]	C	1	24	—
o-Hydroxyamines				
o-Aminophenol[b]	C	2	37	—
2-Amino-1-naphthol hydrochloride[a]	P	20	118	+
2-Amino-1-naphthol hydrochloride[a] (recrystallized Sn^{++})	P	3	68	—
1-Amino-2-naphthol hydrochloride[a]	P	10	36	+
1-Amino-2-naphthol hydrochloride[a] (recrystallized Sn^{++})	P	7	30	+
3-Amino-2-naphthol[b]	P	2	33	—
2-Amino-1-naphthol glucosiduronic acid[c]	C	3	17	?+
2-Amino-1-methoxy-naphthalene[b]	C	13	31	+
2-Dimethylamino-1-methoxynaphthalene hydrochloride[a]	P	2	33	—
4-Hydroxy-3-amino-diphenyl hydrochloride[b]	C	10	36	+
3-Hydroxy-4-amino-diphenyl sulphate[a]	P	9	38	+
4'-Nitro-4-amino-3-hydroxydiphenyl hydrochloride[a]	P	3	20	?+
2-Amino-4 : 5-dimethyl-phenol[c]	C	5	18	+
3-Hydroxykynurenine[c]	C	6	25	?+
3-Hydroxyanthranilic acid[a]	C	8	40	+
3-Hydroxyanthranilic acid[a]	P	1	22	—
2-Amino-3-hydroxy-acetophenone[c]	C	4	14	?+
Aromatic amines				
2-Naphthylamine[a]	P	8	89	?+
4-Aminodiphenyl[a]	P	3	35	?+
2-Anthramine[b]	P	7	46	+
2-Aminofluorene[b]	C	6	38	?+
Azo compounds				
1-Phenylazo-2-naphthol[a]	P	8	32	+
1-*o*-Tolylazo-2-naphthol[b]	C	12	32	+
Other compounds				
2-Amino-1-naphthyl hydrogen sulphate[a]	P	2	38	—
Sodium 4-amino-3-diphenylyl sulphate[a]	P	1	21	—
8-Hydroxyquinoline[b]	C	6	25	+
8-Hydroxyquinoline[c]	C	3	16	?+
4-Amino-1-naphthol[b]	C	3	39	—
8-Methoxy-4-hydroxy-quinoline-2-carboxylic acid[c]	C	3	17	?+
1 : 2-5 : 6-Dibenzanthracene[c]	C	4	12	+

P: Paraffin wax (m.p. 56° C.)
C: Cholesterol

— indicates inactive
+ indicates active
?+ indicates probably active but not statistically significant

[a] Bonser, Bradshaw, Clayson & Jull, 1956
[b] Bonser, unpublished results
[c] Allen, Boyland, Dukes, Horning & Watson, 1957

TABLE IV. AROMATIC AMINES WITHOUT SUBSTITUENTS IN THE *PARA* POSITION, TESTED FOR ACTIVITY AT ALL SITES

Compound	Species	Tumours	Survivors	Latent period (years)	Active isomer	Reference
2-Acetamidodiphenyl	Rat	3 (uterus)	11	1¾–2½	4-Acetamidodiphenyl	Morris, Velat & Wagner (1957)
1-Acetamidofluorene	Rat	1 (adrenal) 2 (pituitary) 1 (uterus) 1 (ovary)	17	¾–1¾	2-Acetamidofluorene	Morris (1955)
4-Acetamidofluorene	Rat	None	17	—	2-Acetamidofluorene	Morris (1955)
9-Anthramine	Rat	None	—	—	2-Anthramine	Bielschowsky (1946)
Aniline	Dog	1 (hepatoma) 1 (bladder papilloma)	4	10–10½	—	Bonser (unpublished findings)
1-Naphthylamine*	Dog	1 (bladder papilloma)	3	8¾	2-Naphthylamine	Bonser (unpublished findings)

*** Containing some 2-naphthylamine**

but there is also evidence that the parent amines are locally active in varying degrees. Three explanations are possible. The amines may be locally metabolized in the tissues to *o*-hydroxyamines. This does not seem likely (cf. Gutmann & Peters, 1957) and should not be considered seriously until biochemical evidence of the presence of suitable enzymes in the bladder epithelium is established. Alternatively, both the amine and the *o*-hydroxyamine may be capable of undergoing a carcinogenic reaction with the tissue, and possibly the *o*-hydroxyamine is in some cases (e.g. 2-amino-1-naphthol) more effective than the amine. If Boyland's suggestion (Boyland & Watson, 1956) that the *o*-hydroxyamines exert their influence through their power of chelation is correct, the above explanation cannot be accepted, as aromatic amines normally do not chelate. Boyland supported his idea by the demonstration that 8-hydroxyquinoline, a potent chelating agent, induced 4 carcinomata in 13 mice. Finally the amine might initiate carcinogenesis not only by its conversion to the *o*-hydroxyamine but also by reaction either with itself or the vehicle. In this way, Bonser, Clayson, Jull & Pyrah (1956) explained the incidence of sarcomata in mice after the subcutaneous injection of aged oily solutions of 2-naphthylamine.

Biochemical studies of the fate of the carcinogenic aromatic amines do not contradict the idea that *o*-hydroxyamines may play a part in the induction of bladder cancer. Thus 2-amino-1-naphthol (unpublished observation), 3-hydroxybenzidine (Bradshaw & Clayson, 1955) and 3-hydroxy-4-aminodiphenyl (Bradshaw, 1958) have been shown to be present in 24-hour collections of urine of dogs dosed with 2-naphthylamine, benzidine and 4-aminodiphenyl respectively. It has, however, not been possible to demonstrate the presence of these metabolites in freshly voided urine. Weisburger, Weisburger & Morris (1957) have shown that 1- and 3-hydroxy-2-acetamidofluorene are present in the urine of rats but not guinea-pigs treated with 2-acetamidofluorene-9-^{14}C, and Daniel (1956) has detected 1-amino-2-naphthol in the urine of rabbits fed 1-phenylazo-2-naphthol.

It was originally suggested (Bonser, Clayson & Jull, 1951) that the free *o*-hydroxyamines might arise by enzymic hydrolysis of their sulphate esters. Boyland, Manson, Sims & Williams (1956), however, demonstrated that many of the *o*-hydroxyamine sulphates were not hydrolysed by sulphatases. They suggested that the glucuronide and phosphate esters were the precursors of the slowly liberated *o*-hydroxyamines. The position has been further complicated by the discovery that the amino group is conjugated in the animal body with glucuronic and sulphuric acids. Boyland (1956) stated that 2-amino-1-naphthyl hydrogen sulphate-*N*-glucosiduronate is not hydrolysed to free 2-amino-1-naphthol by sulphatase, but no investigation on the corresponding sulphamate appears to have been reported.

The *o*-hydroxyamine hypothesis remains for the present an extremely valuable theory for the prediction of compounds likely to be carcinogenic either to the experimental animal or to man. It is not possible to predict the site of election of tumours in the experimental animal and therefore it is unsafe to presume that bladder tumours are the only neoplasms likely to arise in men exposed to these hazards. As both the amines and the *o*-hydroxyamines have been found to be locally carcinogenic to the mouse bladder it is not possible to state categorically which is the effective agent. The absence of skin tumours in the dyestuffs industry indicates that the amines may play the less important role, whereas the skin tumours obtained by painting 2-anthramine (Bielschowsky, 1946) or 2-aminofluorene (Morris, Dubnik & Johnson, 1950) suggest an important role for the amines. Gutmann & Peters (1954, 1956) have shown that the degree of tissue-binding between 2-acetamidofluorene and liver slices is much reduced under conditions where hydroxylation (i.e., metabolism) is inhibited. Only the accumulation of further evidence can help to distinguish the respective parts played by these compounds.

5. Spontaneous Bladder Tumours

The wide-spread use of aromatic amines and azo dyestuffs (for example as food colorants) will in all probability raise the incidence of bladder and other cancer in the general population. There are, however, many so-called spontaneous bladder cancers which cannot reasonably be attributed to these causes. Boyland has made an interesting contribution towards the

discovery of their aetiology.[2] Dunning, Curtis & Maun (1950) showed that a diet containing casein hydrolysate and DL-tryptophan precipitated bladder tumours in rats dosed with 2-acetamidofluorene, whereas a diet containing casein itself was ineffective, although the usual tumours of the liver, acoustic duct and other organs were obtained in all groups. Boyland, Harris & Horning (1954) repeated this observation, but their experiment is less convincing than that of the earlier workers because their bladder tumours appeared after all the rats in the group where the diet contained casein had died. Boyland & Watson (1956) then argued that the urinary metabolites of tryptophan might play some part in precipitating the bladder tumours and went on to test these compounds by bladder implantation. They found that 3-hydroxyanthranilic acid induced more tumours than were found in the controls (Table V), and they suggested that this metabolite might be responsible for " spontaneous " bladder tumours in man. It appears to us that a similar explanation might account for the occasional tumours found in mice implanted with pellets of paraffin wax or cholesterol alone, the pellet acting as the promoting agent to endogenous carcinogens by its presence as a foreign body.

TABLE V. THE CARCINOGENIC ACTIVITY OF 3-HYDROXYANTHRANILIC ACID BY BLADDER IMPLANTATION

Compound	Base	Survivors	Carcinomata
None	Paraffin [a]	9	0
	Paraffin [b]	13*	0
3-Hydroxyanthranilic acid	Paraffin [a]	19	3
	Paraffin [b]	22*	1
	Paraffin [c]	22*	0
None	Cholesterol [a]	8	0
	Cholesterol [b]	24*	1
3-Hydroxyanthranilic acid	Cholesterol [a]	14	4
	Cholesterol [b]	40*	8

[a] Boyland & Watson, 1956
[b] Allen, Boyland, Dukes, Horning & Watson, 1957
[c] Boyland, personal communication
* Includes the mice already described by Boyland & Watson [a]

6. Conclusions and Future Prospects

The experimental induction of bladder cancer was last reviewed in this journal by Bonser (1947). Since then, the systemic testing of carcinogens has continued unabated, the dog often being the favoured species for substances thought to be hazardous to man, and the rat, mouse and rabbit for the more wide-spread investigation of compounds of theoretical interest. The accepted bladder carcinogens, i.e., the aromatic amines and the azo compounds, have not been shown to induce bladder cancer in the cat, ferret or guinea-pig on the few occasions on which these species have been tested. 2-Acetamidofluorene, however, has been shown to induce tumours at sites other than the bladder in the cat (Harding, 1947).

The most important advance of the past decade has been the development of methods for testing carcinogens by their local application to the bladder epithelium. The technique of bladder implantation, in particular, has enabled chemical hypotheses of the mode of induction of bladder tumours to be put to biological test. The suggestion that aromatic amines are carcinogenic by virtue of their metabolism has been supported by the results of testing *o*-hydroxyamines, using the technique of bladder implantation, although it has also been found that some aromatic amines are locally active. Further evidence will be required before the relative importance of these bases or their metabolites in the induction of bladder cancer can be assessed.

Biochemical investigation of the urinary metabolites of some bladder carcinogens has shown that *o*-hydroxyamines are present in 24-hour samples of urine. As these metabolites have not been detected in freshly voided urines it will be necessary to determine their urinary precursors with more certainty than at present. It will also be necessary to investigate the metabolism of amines which have not been shown to induce cancer to determine whether or not these are converted to *o*-hydroxyamines.

The advent of radiochemical techniques will greatly facilitate biochemical studies of the bladder carcinogens. The use of isotopically-labelled aromatic amines should make possible the investigation of the mode of interaction of the carcinogen with bladder epithelium, an aspect of carcinogenesis which is assuming great importance in the light of recent theories (Green, 1954).[3] The bladder epithelium is not likely to be the usual tissue of choice for the study of carcinogen–tissue interaction, because it is so much less abundant than the skin epidermis and liver. Goldblatt and his colleagues have, however, observed in the rat and rabbit that the bladder and the red cells, of all the tissues studied, are the only ones capable of retaining systemically administered 2-naphthylamine-5 : 8-^{14}C in detectable amounts for appreciable periods (Henson, Somerville, Farquharson & Goldblatt, 1954; Somerville, Henson, Cooke, Farquharson & Goldblatt, 1956).

It appears to us that this kind of approach is likely to be of the utmost importance in the further elucidation of the mechanism of action of bladder carcinogens.

[1] See also Boyland, p. 153 of this number of the Bulletin.—ED.

[2] See also Green, p. 101 of this number of the Bulletin.—ED.

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THE BIOCHEMISTRY OF CANCER OF THE BLADDER

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1 Mechanism of carcinogenesis by 2-naphthylamine
2 Carcinogenic action of tryptophan metabolites
3 Enzymes in bladder carcinogenesis
4 Inhibition of β-glucuronidase
References

The induction of cancer of the bladder by aromatic amines is of particular interest to biochemists because of the metabolic processes involved. Cancer of the bladder is an occupational disease which occurs frequently among men employed in the manufacture of certain aromatic amines, including 2-naphthylamine (I), benzidine (II) (Case, Hosker, McDonald & Pearson, 1954), and 4-aminodiphenyl (III) (Melick, Escue, Naryka, Mezera & Wheeler, 1955).[1] The manufacture of 2-naphthylamine has ceased in Britain because of the hazard involved. The carcinogenic effect of these aromatic amines appears to be specific for the urinary bladder since, although the skin, lungs and alimentary tract of chemical workers were exposed to the amines, the increased cancer incidence appeared to be confined to the bladder. When bladder cancer was first recognized as an industrial disease, Rehn (1895) suggested that the action was due to some material excreted in the urine. This organ-specificity suggests that the carcinogenic action is due to metabolites of the amines excreted in urine, rather than to the amines themselves, and this has been confirmed by many investigations.

1. Mechanism of Carcinogenesis by 2-Naphthylamine

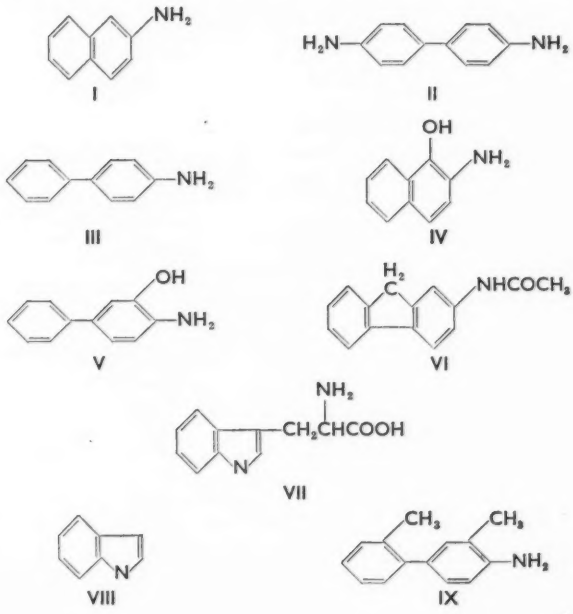
Cancer of the bladder is not usually induced in laboratory rodents (except for certain strains) by treatment with aromatic amines, but Hueper, Wiley & Wolfe (1938) and Bonser (1944) showed that 2-naphthylamine induces bladder cancer when administered to dogs. Some amines (e.g., 4-aminodiphenyl (III) and 3 : 2′-dimethyl-4-aminodiphenyl (IX) induce cancer of the bladder in dogs (Walpole, Williams & Roberts, 1954) but cancer of the intestine in rats (Walpole, Williams & Roberts, 1952). Other amines which induce bladder cancer when given to dogs but liver cancer in rats are 2-acetamidofluorene (VI) (Morris & Eyestone, 1953), *o*-aminoazobenzene and 4-dimethylaminoazobenzene (Nelson & Woodard, 1953).

Cancer of the bladder is, however, induced in rats when they are dosed with the carcinogen 2-acetamidofluorene (VI) and maintained on a diet containing added tryptophan (VII) (Dunning, Curtis & Maun, 1950) or indole (VIII) (Dunning & Curtis, 1957). Without these additions to the diet the carcinogen usually induces cancer in other sites such as the liver or acoustic duct. 2-Naphthylamine or benzidine do not induce cancer of the bladder in rats even when they are kept on a diet supplemented by tryptophan (Boyland, Harris & Horning, 1954).

Bonser, Clayson, Jull & Pyrah (1952) (and see Bonser, Clayson & Jull, 1958) developed the experimental procedure of implantation of paraffin wax pellets containing suspected carcinogens into the bladders of mice. When tested by this method neither 2-naphthylamine (I) nor 4-aminodiphenyl (III) induced cancer in significant numbers of mice, but intermediary metabolites of these amines—2-amino-1-naphthol (IV) and 4-amino-3-hydroxydiphenyl (V), which are both *o*-aminophenols—induced cancer in a considerable proportion of mice (Bonser, Bradshaw, Clayson & Jull, 1956). In this technique the possibilities of metabolic change of the applied carcinogen are much less than when the carcinogen is administered orally or by parenteral injection, because the applied material diffuses directly to the bladder epithelium. The carcinogenic hydrocarbons such as 1 : 2-5 : 6-dibenzanthracene induce cancer when administered by this method, which indicates that they act without undergoing previous metabolic change. On the other hand some amines must be metabolized to exert their carcinogenic action. Whether all aromatic amines need to undergo metabolic change before producing their biological effects is not yet proved.

Boyland & Watson (1956) modified the technique of bladder implantation by using pellets of cholesterol made under pressure (to avoid heating the substances under test) and by tying off the bladder after insertion of the pellet (instead of sewing up the lumen).

Wiley (1938) found that dogs treated with 2-naphthylamine excrete 2-amino-1-naphthol as the sulphuric ester (Table I, no. 4) in the urine, and Hueper (1938) showed that an impure preparation of the 2-amino-1-naphthol (IV) induced cancer on injection into mice. Later Manson & Young (1950) isolated the same metabolite from the urine of rats. Dobriner,



[1] For further discussion of this subject see the papers in this Bulletin by Bonser *et al.* (p. 146), Goldblatt (p. 136).—ED.

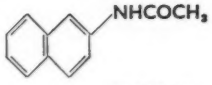
Hoffman & Rhoads (1941) identified 2-acetamidonaphthalene (Table I, no. 1) and 2-acetamido-6-naphthol (Table I, no. 10) as the metabolites of 2-naphthylamine in monkeys, rabbits and rats. These early observations presented a simple view of the species differences, and Boyland & Weigert (1947) drew attention to the difference in the excreted metabolites of the susceptible species and resistant species. More metabolites of 2-naphthylamine have been isolated or identified by Boyland & Manson (1955, 1957, 1958 and unpublished observations) and Boyland, Manson & Orr (1957) using techniques of paper chromatography and column chromatography. Of the 15 metabolites listed in Table I the last three have not yet been isolated but have been identified on chromatograms. The 2-amino-1-naphthol that Bonser *et al.* (1956) have shown to be carcinogenic in the mouse bladder was never detected in the fresh urine of rats, rabbits or dogs to which 2-naphthylamine had been administered, although the 2-amino-1-naphthyl sulphate and glucosiduronate were generally present. Some of the isolated metabolites have been tested for carcinogenic activity in the mouse bladder by Allen, Boyland, Dukes, Horning & Watson (1957).

Consideration of the properties of the identified metabolites in the light of knowledge of the carcinogenic action of some of the known derivatives of aromatic amines indicated that only 2-amino-1-naphthyl glucosiduronic acid (Table I, no. 5) should be the cause of bladder cancer (Boyland, 1956). The experimental data indicate that the carcinogenic metabolites of aromatic amines are *o*-aminophenols, so that the 2-amino-1-naphthol derivatives only should be carcinogenic. In agreement with this is the finding that 2-amino-6-naphthol, 2-amino-6-naphthyl hydrogen sulphate and 2-acetamido-6-naphthyl hydrogen sulphate are not carcinogenic in the mouse bladder (Allen, Boyland & Dukes, unpublished observations). For this reason the derivatives of 2-amino-6-naphthol and 2-acetamido-6-naphthol (Table I, nos. 8, 9, 10, 11) are unlikely to be active. The *N*-substituted naphthylamine derivatives 2-naphthyl sulphamate and 2-naphthylamino-*N*-glucosiduronic acid (Table I, nos. 2, 3) are not carcinogenic, in agreement with the fact that enzymic hydrolysis could liberate only 2-naphthylamine which is not itself carcinogenic when implanted directly in the bladder. The metabolites, which are derivatives of 2-amino-5:6-dihydroxynaphthalene (Table I, nos. 13, 14, 15), like the 6-hydroxy derivatives could not yield 2-amino-1-naphthol.

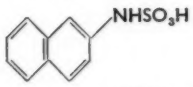
2-Amino-1-naphthyl hydrogen sulphate (Table I, no. 4), which was the first metabolite to be isolated, yields the carcinogenic 2-amino-1-naphthol on acid hydrolysis. This compound is, however, not hydrolysed by the urinary arylsulphatase (Boyland, Manson, Sims & Williams, 1956) although this enzyme hydrolyses those phenyl sulphuric esters (e.g., sodium 1-naphthyl sulphate, fig. 1) which do not have basic amino groups in the *ortho* position. 2-Amino-1-naphthyl

TABLE I. METABOLITES OF 2-NAPHTHYLAMINE

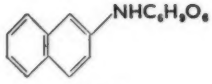
1. 2-Acetamidonaphthalene



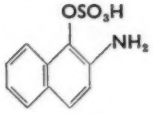
2. 2-Naphthyl sulphamic acid



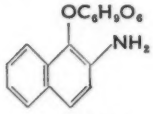
3. 2-Naphthylamino-*N*-glucosiduronic acid



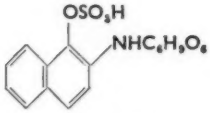
4. 2-Amino-1-naphthyl hydrogen sulphate



5. 2-Amino-1-naphthyl glucosiduronic acid

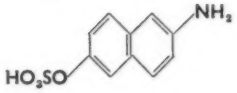


6. 2-Amino-1-naphthyl hydrogen sulphate *N*-glucosiduronic acid

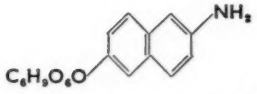


7. 2-Amino-6-naphthol

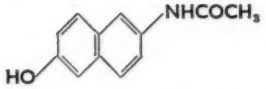
8. 2-Amino-6-naphthyl hydrogen sulphate



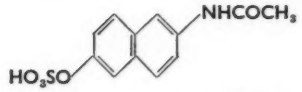
9. 2-Amino-6-naphthyl glucosiduronic acid



10. 2-Acetamido-6-naphthol

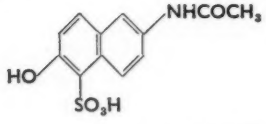


11. 2-Acetamido-6-naphthyl hydrogen sulphate

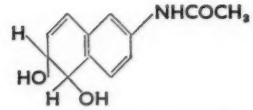


12. 2-Acetamido-6-naphthyl glucosiduronic acid

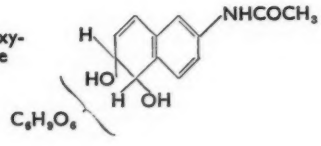
13. 2-Acetamido-5:6-dihydroxy-naphthalene-5-hydrogen sulphate



14. 2-Acetamido-5:6-dihydroxy-5:6-dihydronaphthalene

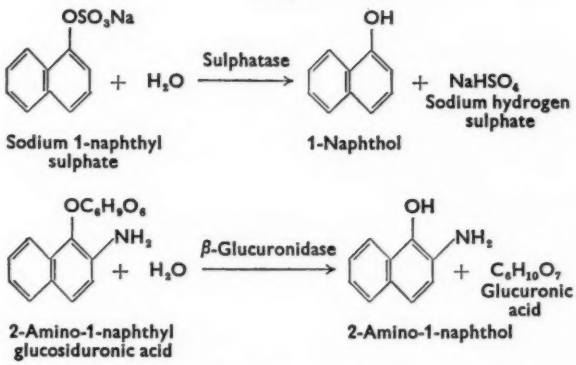


15. 2-Acetamido-5:6-dihydroxy-5:6-dihydronaphthalene glucosiduronic acid



hydrogen sulphate is apparently not carcinogenic when tested in the bladders of mice (Bonser *et al.* 1956). The corresponding 2-amino-1-naphthyl glucosiduronic acid (Table I, no. 5) is, however, hydrolysed to 2-amino-1-naphthol by the urinary β-glucuronidase (fig. 1) and has induced tumours when implanted in the mouse urinary bladder (see Plate III, fig. A) (Allen *et al.* 1957).

FIG. 1

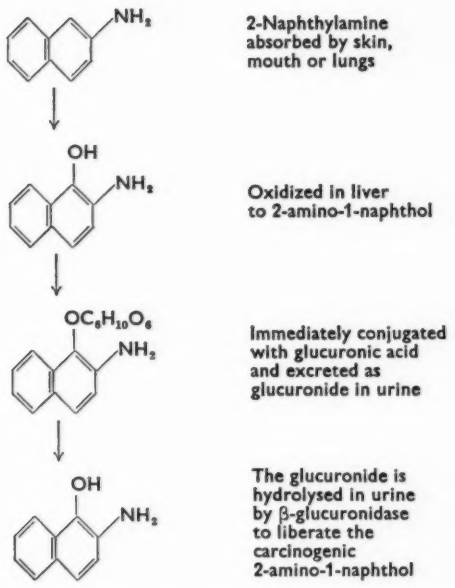


The following suggested mechanism of carcinogenesis indicated schematically in fig. 2 would explain the fact that 2-naphthylamine induces cancer of the bladder but does not induce cancer in other organs or tissues. The 2-naphthylamine is oxidized in part to 2-amino-1-naphthol in the liver but is immediately conjugated either with sulphuric acid or with glucuronic acid (cf. Booth, Boyland & Manson, 1955). The rates of the conjugation reactions are each ten times as great as the rate of formation of the 2-amino-1-naphthol, so that the concentration of the free 2-amino-1-naphthol in the liver is minimal. The 2-amino-1-naphthyl glucosiduronic acid is excreted in the urine and not reabsorbed, so that the concentration in urine is higher than in tissues or body-fluids. In the urine it is exposed to the action of the soluble excreted β-glucuronidase at a pH (5–6) which is nearer to the optimum pH (4.5) of this enzyme than are the neutral body-fluids and tissues. While the urine remains in the bladder, some 2-amino-1-naphthol is liberated and this penetrates the bladder mucosa. In addition to the factors of concentrations of 2-amino-1-naphthyl glucosiduronic acid and β-glucuronidase activity, the carcinogenic process would be favoured by acid conditions of urine (up to the optimum of pH 4.5) and by an increase in the time during which the urine remains in the bladder. The carcinogenic action is therefore dependent on the two factors: (i) the *o*-aminophenol esters or ethers and (ii) the activity of hydrolysing enzymes such as β-glucuronidase.

2. Carcinogenic Action of Tryptophan Metabolites

The bladder cancers that develop among chemical workers in the dyestuffs industry following exposure to aromatic amines do not differ in their natural history or in pathology from the tumours that arise in the general population. These latter tumours, by analogy with those associated with the industrial hazards might, therefore, be due to some carcinogen liberated in the urine from a product of intermediary metabolism. A number of *o*-aminophenols are now known to induce bladder cancer, and three *o*-aminophenols—3-hydroxykynurenine, 3-hydroxyanthranilic acid and 2-amino-3-hydroxyacetophenone—(see fig. 3 and Plate III, figs. B, C), which are metabolic products of tryptophan, occur in human urine. These metabolites and 8-methoxykynurenic acid (the methyl ether of xanthurenic acid) have been tested by the bladder implantation procedure and found to be carcinogenic (Allen *et al.* 1957). These substances are therefore carcinogens which are products of endogenous metabolism and might be the cause of cancer now considered as spontaneous. Other tryptophan metabolites such as 5-hydroxytryptamine, kynurenic acid and xanthurenic acid were inactive when tested as carcinogens in the mouse bladder.

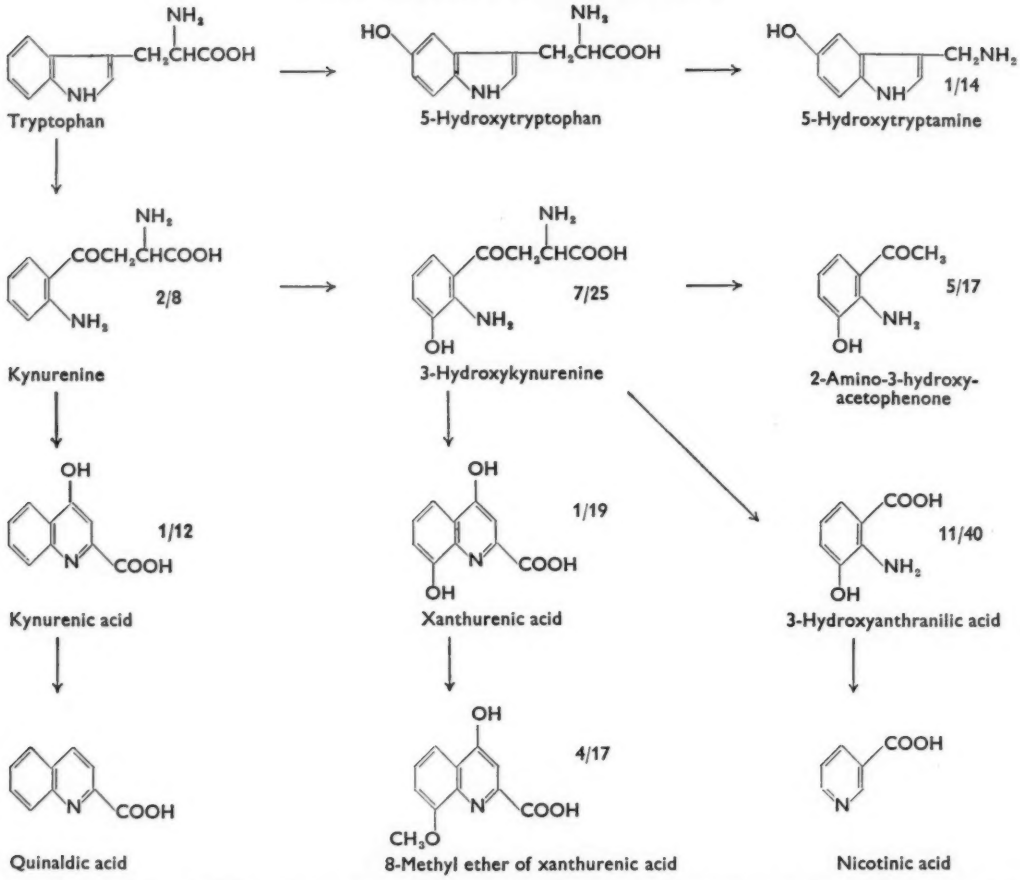
FIG. 2. SCHEMATIC REPRESENTATION OF THE METABOLISM OF 2-NAPHTHYLAMINE LEADING TO THE LIBERATION OF THE CARCINOGENIC 2-AMINO-1-NAPHTHOL IN URINE



Methods of estimating the tryptophan metabolites in human urine, first by column chromatography (Boyland & Williams, 1956a; Brown, 1957) and later by spot areas on paper chromatograms (Boyland & Williams, 1956b), have been used. Both methods were applied to urine from normal subjects and bladder-cancer patients under normal conditions and following administration of a standard dose of 2 g. tryptophan which increases the excretion of tryptophan metabolites. The 3-hydroxykynurenine and 3-hydroxykynurenine sulphate excreted generally amounted to less than 3 mg./l. of urine. The quantities of 3-hydroxyanthranilic acid were greater in patients with bladder cancer (5–15 mg./l.) than in normal subjects (0–6 mg./l.) or in patients with cancer of sites other than the bladder (0–10 mg./l.). The excreted 3-hydroxyanthranilic acid may therefore be a causative factor in cancer of the bladder in man.

The carcinogenic action of these tryptophan metabolites might be related to the mutagenic effect of tryptophan which has been observed in drosophila (Plaine & Glass, 1955) and in bacteria (Novick & Szilard, 1951; Green, Dodd & Radike, 1955).

FIG. 3. METABOLISM OF TRYPTOPHAN



In the numbers printed as fractions at the side of some formulae, the numerator refers to the number of mice with tumours, and the denominator to the number of mice surviving for 30 weeks or more. The incidence of tumours in the control animals implanted with cholesterol pellets was 1/24.

Bladder cancer in men not employed in the manufacture of aromatic amines may be due to endogenous carcinogens, but the exogenous bladder carcinogens have been very widely distributed in industry during recent years. Carcinogenic amines were used in the manufacture of rubber, and articles made of rubber are widely used in the electrical, automobile and other industries. The incidence of bladder cancer differs from that of cancer of some other sites (except the lungs) in that the incidence is increasing in Great Britain (Case, 1956) and in Copenhagen (Clemmesen & Nielsen, 1956). This apparent increase may be due to improvement in diagnosis, but it is difficult to understand why diagnosis of cancer of the bladder should have improved more than that of cancer of other sites. The increase might be due in part to wide-spread exposure to carcinogenic substances.

3. Enzymes in Bladder Carcinogenesis

The carcinogenic phenolic tryptophan metabolites are (like other aminophenols) excreted as conjugates either with sulphuric acid or glucuronic acid. The conjugates would be hydrolysed by the appropriate enzyme, sulphatase or β-glucuronidase, present in almost all urines, but in higher concentrations in patients with cancer of the bladder (cf. Table II based on data in Boyland, Wallace & Williams, 1955; Boyland, Gasson & Williams, 1957). Whereas most of the β-glucuronidase activity of tissues is confined to cell particles (e.g., the lysosomes described by Duve, Pressman, Gianetto, Wattiaux & Appelmans, 1955) and not freely available for action on glucosiduronic acids, the urinary β-glucuronidase appears to be in solution and available for hydrolysis of glucosiduronic acids. Thus, in patients with bladder cancer, both the factors which might cause the disease—the *o*-aminophenol conjugate and the enzyme—are present in raised concentrations.

This suggested series of metabolic processes, whereby a carcinogen is liberated in the urine contained in the urinary bladder, represents an Achilles heel in the defences of the body against toxic substances. The detoxified carcinogen concentrated in the urine is activated by enzymes present in urine, probably excreted adventitiously.

TABLE II. β-GLUCURONIDASE AND ARYLSULPHATASE ACTIVITIES IN URINES OF DIFFERENT TYPES OF PATIENTS

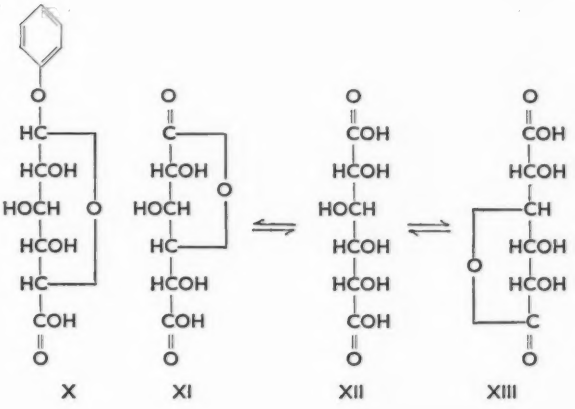
Nature of disease	β-Glucuronidase			Arylsulphatase		
	Number of cases	Units/ml. (range)	Number of cases with values above normal range	Number of cases	Units/ml. (range)	Number of cases with values above normal range
None	25	0.1–1.2	—	25	0–0.82	—
Cancer of bladder: single tumours	25	1.1–7.6	24	43	0.24–3.0	20
Cancer of bladder: multiple tumours	39	0.8–7.9	35	41	0.52–5.0	33
Cancer of bladder: with new tumours or recurrence following treatment	19	1.1–7.4	18	15	0.4–3.0	6
Cancer of bladder, but free of tumours following treatment	16	0.9–7.2	14	16	0.23–3.5	12
Cancer of kidney	9	0.3–3.5	2	6	0.35–0.90	0
Cancer of colon or rectum	10	0.3–1.7	3	2	0.8–1.7	1
Cancer of breast	15	0.3–2.6	8	2	0.6–1.3	1
Urinary infection	22	0.2–1.5	1	16	0–2.0	8

An analogous mechanism for cancer induction might occur in the intestine. Any carcinogen inactivated in the liver may be excreted in the bile as well as in urine. In the alimentary tract the detoxified carcinogen would be subjected to hydrolytic enzymes secreted into the tract or produced by the intestinal flora. As the intestinal contents are concentrated during their passage down the gut the enzymic hydrolysis would increase and be maximal in the stationary faeces. In agreement with this hypothesis is the fact that 3 : 2′-dimethyl-4-aminodiphenyl induces bladder cancer in dogs but intestinal tumours in rats. Cancer of the intestine and rectum have similar ratios of incidence in the sexes to cancer of the bladder, being three times more common in men than in women (Case, 1956).

4. Inhibition of β-Glucuronidase

The activity of the urinary enzymes can be reduced by oral administration of sodium phosphate, an inhibitor of sulphatase, and of glucosaccharo-1→4-lactone, the most potent inhibitor of β-glucuronidase (Levvy, 1952). Glucosaccharo-1→4-lactone (XI), which is a lactone of saccharic acid (XII), is a competitive inhibitor of β-glucuronidase, presumably because of the structural resemblance to phenyl glucosiduronic acid (X). The isomeric glucosaccharo-6→3-lactone (XIII), which differs from glucosiduronic acids in the steric relationships of the hydroxyl groups, does not inhibit β-glucuronidase. If 1 g. of saccharo-1→4-lactone is taken three times daily, sufficient is excreted in the urine to reduce the apparent β-glucuronidase activity by 80–90% (Boyland, Wallace & Williams, 1957). A group of patients who have had malignant tumours of the bladder removed are at present under continuous treatment with saccharo-1→4-lactone in the hope of reducing the chance of development of new tumours. If the lag or latent period between the application of the carcinogenic stimulus and the appearance of cancer is of the order of ten or twenty years, then the stimulus which induces tumours that arise after operation may have been delivered long before the treatment began. There is, however, a possibility that the cancers of the bladder induced by aminophenols are (at least during the early stages) dependent on the continued presence of the carcinogen (e.g., 3-hydroxyanthranilic acid). In this case the tumours might be restrained by inhibition of enzymes, which would reduce the concentration of the carcinogen to levels below which the dependent tumour can grow. This prophylactic treatment should be combined with other measures which would reduce the effect of excreted carcinogens—such as drinking of large volumes of liquid to dilute the urine, and frequent micturition, so that the urinary enzymes act on the " detoxified " carcinogens in the bladder for short periods only.

Prophylactic treatment with saccharo-1→4-lactone would, however, probably be of more value in preventing bladder cancer following accidental exposure to known carcinogenic amines. Stringent precautions are taken in factories in which



benzidine, 1-naphthylamine and 4-aminodiphenyl are manufactured, so that personnel are not usually exposed to the amines. If workers were contaminated as the result of an accident, then treatment with saccharo-1→4-lactone immediately following the exposure for a few days should reduce the risk of development of bladder cancer. Preparations of saccharo-1→4-lactone should be available in the factories for treatment of any workers exposed under these conditions. Treatment with saccharo-1→4-lactone should prevent the carcinogenic action of 2-naphthylamine or 4-aminodiphenyl in dogs, and it is hoped that this will be tested experimentally.

If the hypothesis of the origin of bladder cancer leads to control of the disease in men not exposed to any industrial hazard, then the chemical workers who died with this kind of tumour could be considered as comparable with the radiologist martyrs listed by Spear (1956). The knowledge which we have gained from their misfortune may be of value in reducing the incidence of this form of disease.

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* * *

THE BIOCHEMISTRY OF CANCER OF THE BLADDER

(FIGS. A–C)

E. Boyland

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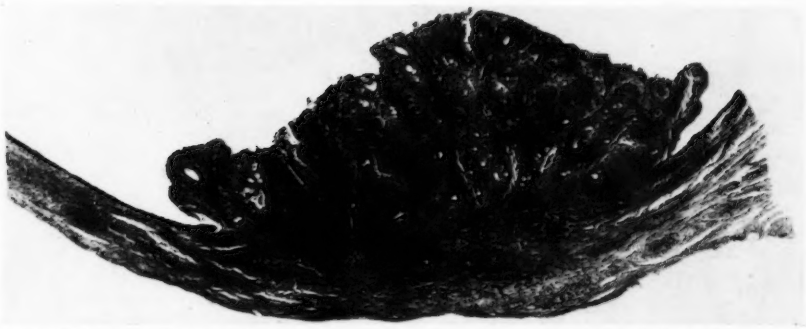

FIG. A. Papilloma from bladder of a mouse 40 weeks after implantation of cholesterol pellet containing 2-amino-1-naphthyl glucosiduronic acid

(magnification × 32)

FIG. B. Section of distended bladder from a mouse 40 weeks after implantation of cholesterol pellet containing 2-amino-3-hydroxyacetophenone

(magnification × 12.5)

FIG. C. Higher magnification of focus of carcinoma from bladder shown in fig. B, showing small invasive focus of carcinoma

(magnification × 50)

Allen et al. *(1957) by permission of British Journal of Cancer*

PLATE IV (FIGS. A—F)

PHOTOMICROGRAPHS SHOWING SARCOMATA INDUCED AT SITE OF INJECTION OF PURIFIED CHOLESTEROL IN OLIVE-OIL

(FIGS. A—F)

I. Hieger

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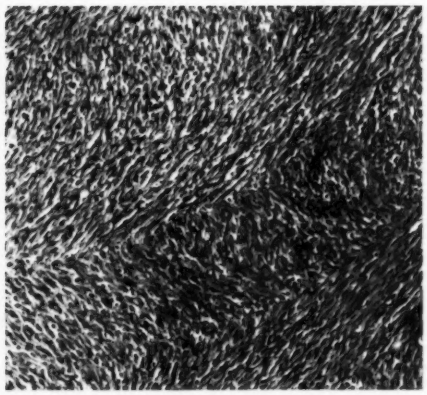

FIG. A

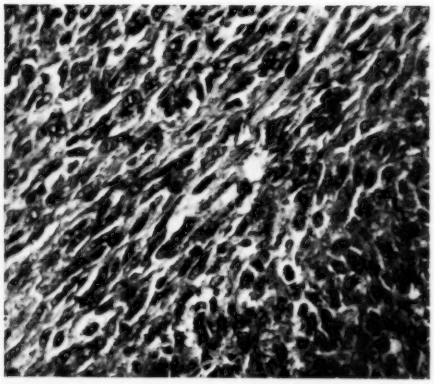

FIG. B

FIG. A. Sarcoma in female stock mouse, which appeared 16 months after commencement of injections.
(magnification × 56)

FIG. B. Same tumour as in fig. A
(magnification × 250

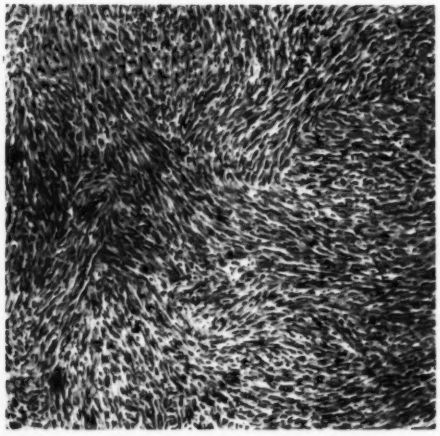

FIG. C

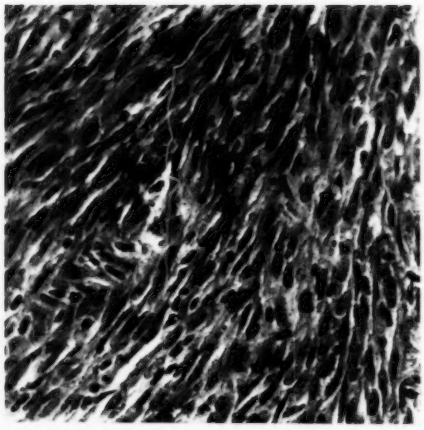

FIG. D

FIG. C. Sarcoma in male stock mouse, which appeared 18 months after commencement of injections.
(magnification × 56)

FIG. D. Same tumour as in fig. C
(magnification × 250)

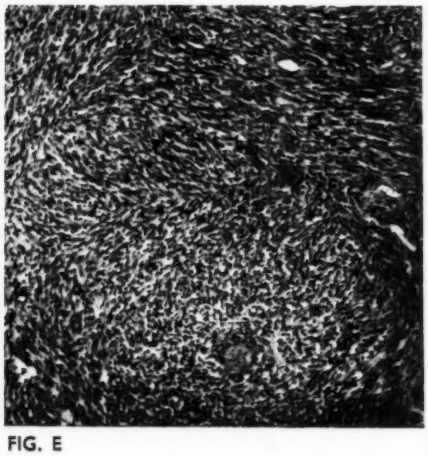

FIG. E

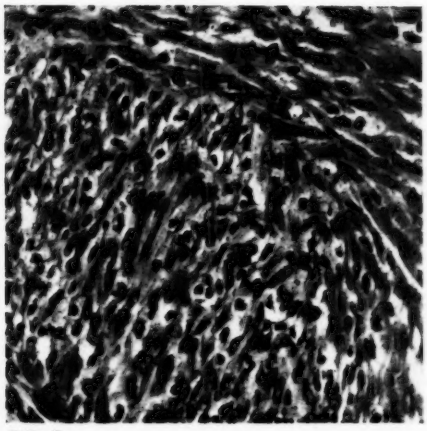

FIG. F

FIG. E. Sarcoma in male stock mouse, which appeared 9 months after commencement of injections.
(magnification × 62)

FIG. F. Same tumour as in fig. E
(magnification × 250)

CHOLESTEROL CARCINOGENESIS

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1 Injection of cholesterol
2 The variation in the incidence of tumours
3 Summary
References

The pioneer work of Shabad (1937) on endogenous carcinogens demonstrated that sarcoma could be induced in mice by the subcutaneous injection of crude lipoid extracts from post-mortem human tissue. His discovery was confirmed by other workers (Des Ligneris, 1940; Hieger, 1940; Steiner, 1941; Sannié, Truhaut, Guérin & Guérin, 1941) who obtained tumours by injecting the unsaponifiable fraction (i.e. the cholesterol-rich fraction) of the livers of human subjects who had died of cancer or from some other cause. The succeeding steps in this investigation gave evidence of the carcinogenic potency of cholesterol itself even after it had been highly purified (Hieger, 1947, 1949, 1957; Hieger & Orr, 1954).[1]

1. Injection of Cholesterol

The technique of purifying and testing cholesterol has been described in detail in the earlier papers, as also have the histological characteristics of the sarcomata produced (see Plate IV, figs. A–F), the results of transplantation and the very low yield of sarcomata (2 in 700 mice) in the control tests on solvents alone. In brief, the experimental technique was as follows:

i. The cholesterol is purified by the bromination method of Schwenk & Werthessen (1952); the product has a very low absorption in u.v. and, judging purity by this criterion, no advantage is gained by using a more elaborate purifying technique where the cholesterol passes through five stages before being finally crystallized.
ii. The mice are kept in groups of five in zinc boxes supplied with hoppers containing standard food pellets and drinking-water in glass bulbs.
iii. The solutions of cholesterol (10%) are prepared by heating cholesterol with olive-oil or lard on the water bath.
iv. The injections (usually 0.25 ml.) are made subcutaneously in the right flank, and are repeated at intervals of three or four weeks until a stable nodule is established; from 3 to 5 injections are sufficient for this purpose.

The results of the tests on cholesterol are detailed in Table I which gives the yield of sarcomata in experiments where the conditions embrace variations in the factors mentioned in the table:

i. The geographical position of the laboratory with consequent alterations of the degree of atmospheric pollution by town smoke.
ii. The possibility of trace contamination by the vapour of benzopyrene.
iii. The strain of mouse.
iv. The average temperature.
v. The solvent used as vehicle for administering the cholesterol (lard at Chester Beatty Research Institute; olive-oil at Pollards Wood Research Station).
vi. The purity of the cholesterol.

2. The Variation in the Incidence of Tumours

The 70 sarcomata obtained in the experiments provide the evidence for the conclusion that cholesterol is a carcinogen: however, it is difficult to find any satisfying explanation of why some batches of mice are resistant to cholesterol.

Table I shows that cholesterol in repeated tests can induce an incidence of sarcomata as high as 14%, yet sometimes yields only a single sarcoma (or even none at all) in a group of 30–60 mice.

Such variations have also been found in the frequency of spontaneous fibrosarcoma in groups of mice in the studies of Dunn, Heston & Deringer (1956). Steiner and his co-workers (Steiner, 1943; Steiner, Stanger & Bolyard, 1947), who tested the carcinogenic activity of the unsaponifiable fraction of liver, also observed fluctuations of response. The conditions in the experiments were as follows. Livers were obtained from 37 human subjects with cancer and from 30 people free of malignant disease. The livers were treated separately by saponifying twice in boiling ethanolic KOH, and extracted with ethylene dichloride. The extract, consisting of the unsaponifiable matter (largely cholesterol), was ground with sesame oil or with tricaprylin, and injected into mice of the C57 strain or of a partly inbred albino stock.

The liver extracts from the cancer cases (37 in number) were tested separately in 37 batches of mice numbering 6–19 members per batch, and similarly with the 30 non-cancer extracts, in batches of 4–21 mice. The 37 cancer extracts induced 12 sarcomata in an initial total of 456 mice, distributed thus: 29 extracts gave no sarcomata, 5 gave 1 sarcoma each, 2 gave 2 each and 1 gave 3 sarcomata in 16 mice.

The 30 non-cancer extracts induced 10 sarcomata in 440 mice: 23 of these extracts gave no sarcomata, 3 gave 1 sarcoma each, 2 gave 2 sarcomata and 1 gave 3 sarcomata in 17 mice.

In the later paper, Steiner and colleagues (Steiner *et al.* 1947) reported that the unsaponifiable fraction from 10 pooled non-cancer livers tested by the same technique (which gave 10 sarcomata in 440 mice) produced 27 sarcomata in 46 C57 mice, an incidence of 59%; this is about 15 times higher than in the earlier experiment. At first sight, the remarkably high activity of this preparation could be explained by the assumption that one of the livers in the pool of 10 contained an exceptionally large amount of carcinogen, just as 1 liver in 37 in the previous experiments gave 3 sarcomata in 17 mice initially, and 1 liver in 30 gave 3 sarcomata in 16 mice.

But the striking similarity between the results of Steiner and the fluctuations in the yield of sarcomata induced by cholesterol, where the carcinogen was a constant factor, suggests rather that the variations were due to important differences not in the composition of the liver extracts but in the sensitivity of the mice.

[1] See also Hieger, *Brit. med. Bull.* 1947, 4, 360.—Ed.

TABLE I. INCIDENCE OF SARCOMATA IN MICE AFTER SUBCUTANEOUS INJECTION WITH CHOLESTEROL

Experiment	Institute*	Room*	Strain	Pure or commercial cholesterol	Number of mice				Number of sarcomata	Incidence in initial number of mice (%)	Incidence calculated on survivors at 12 months (%)
					Initial	Survivors after					
						12 months	18 months	24 months			
a	C.B.		C57	Commercial	108	68	45	10	10	9	14
b	C.B.		Stock	Commercial	45	18	5	3	5	11	28
c	C.B.		MRC	Commercial	20	16	8	2	2	10	12
d	C.B.		C57 + stock	Pure	34	18	11	2	1	3	6
e	P.W.	Non-carcinogen	C57	Commercial	60	28	14	3	1	2	4
f	P.W.	Non-carcinogen	Stock	Commercial	70	38	18	2	3	4	8
g	P.W.	Non-carcinogen	Stock	Commercial	65	57	41	11	3	5	5
h	P.W.	Carcinogen	Stock	Commercial	100	75	45	10	8	8	11
i	P.W.	Carcinogen	CBA	Commercial	100	71	45	2	2	2	3
j	P.W.	Non-carcinogen	C57	Pure	172	126	81	20	5	3	4
k	P.W.	Non-carcinogen	C57	Commercial	30	10	7	5	1	3	10
l	P.W.	Non-carcinogen	C57	Commercial	39	24	16	11	3	8	13
m	P.W.	Carcinogen	C57	Commercial	75	46	26	3	4	5	9
n	P.W.	Carcinogen	Stock	Pure	115	66	22	10	11	10	14
o	P.W.	Non-carcinogen	Stock	Pure	109	All dead in less than 12 months (epidemic)			3	3	Probably much more than 3
p	P.W.	Non-carcinogen	Stock	Commercial	53	27	11	0	0	0	0
q	P.W.	Non-carcinogen	C57	Commercial	50	38	32	23	0	0	0
r	P.W.	Non-carcinogen	Stock	Pure	53	25	12	2	0	0	0
s	P.W.	Non-carcinogen	C57	Pure	50	47	35	22	3	6	6
t	P.W.	Low-temperature	C57	Commercial	46	32	23	12	5	11	16
u	P.W.	Low-temperature	Stock	Commercial	40	19	5	0	0	0	0

*** Key to Table I**

C.B. : Chester Beatty Research Institute, London

P.W. : Pollards Wood Research Station (in Buckinghamshire, 20 miles out of London)

Non-carcinogen room: carcinogens of conventional type (e.g. 3:4-benzopyrene) were neither used nor kept in this room.

Carcinogen room: in this room, besides mice injected with cholesterol, were other series of mice undergoing treatment with 3:4-benzopyrene.

Low-temperature room : this room faces north ; temperature not below 40° F. (4.5° C.), but otherwise follows outside temperature; mice are supplied with water only as present in cabbage leaf.

Since the experiments detailed in Table I have largely eliminated the effects of changes in the composition of the injected material, the presence of traces of hydrocarbon-carcinogens in the air, the strain of mouse, the purity of the cholesterol and the solvent used as the vehicle for injection, there seems at present no obvious alternative to the explanation that the fluctuations in response are due to some environmental or congenital factors.

3. Summary

The carcinogenic activity of cholesterol has been demonstrated. Seventy sarcomata were induced in 1,434 mice (initially) by the injection subcutaneously of oily solutions of cholesterol. The incidence is variable; it fluctuates between about 14% and zero. The experiments described in this paper suggest that such variations are probably due to susceptibility differences in the mice, of congenital or environmental origin.

ACKNOWLEDGEMENTS

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SOME DYNAMIC ASPECTS OF CHEMICAL CARCINOGENESIS

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1 Metabolism and excretion of carcinogens
2 Growth-inhibitory action of carcinogens
3 Enzyme inhibition and tissue metabolism
References

Following the discovery of the carcinogenic action of pure aromatic hydrocarbons, the increasing number and diversity of types of chemical carcinogens, e.g. aromatic amines, azo compounds, aliphatic and aromatic *N*-dichloroethylamines (nitrogen mustards), urethanes, epoxides, etc., has made it clear that carcinogenesis cannot be associated directly with any one type of chemical structure or reactivity.

A theory of a relationship between electronic structure and carcinogenic activity of aromatic molecules has been put forward by the French school of theoretical chemists Dr and Mme Daudel, Dr and Mrs Pullman and their collaborators (Pullman, 1945, 1946, 1947; Pullman & Pullman, 1946, 1948). It has been reviewed by Coulson (1953) and by Pullman & Pullman (1955).

Evidence for a direct action of carcinogenic azo compounds on liver proteins has been obtained by Miller & Miller (1952, 1953). This, together with studies of binding of carcinogenic hydrocarbons with skin proteins (Miller, 1951) and quantitative studies of such protein-binding by using ^{14}C-labelled carcinogenic hydrocarbons (Wiest & Heidelberger, 1953; Heidelberger & Moldenhauer, 1956), has led to a "protein deletion" hypothesis of carcinogenesis (Miller & Miller, 1955).

However, I would like to suggest here that carcinogenesis by certain types of chemicals, including aromatic hydrocarbons, azo compounds and aromatic amines, may be thought of as essentially a dynamic process in which we should consider the carcinogen as a foreign substance being subjected to the normal metabolic processes of the so-called detoxication mechanisms (Williams, 1947). It is conceivable that, with carcinogenic substances, some stage of this detoxication process may go wrong, with the result that a toxic product is liberated in the cells of certain organs. These toxic products may act in various ways, possibly by direct action on chromosome material (Boyland, 1952) or also by inhibiting certain enzyme systems—particularly those concerned with oxidative metabolism and respiration.

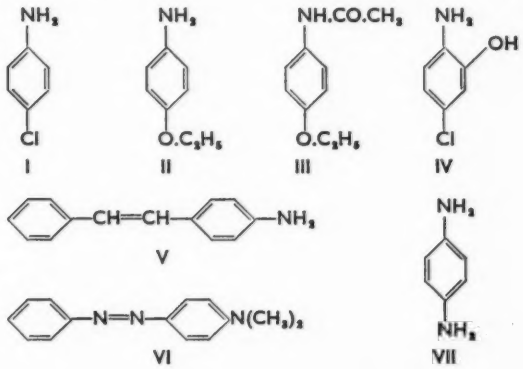
A brief examination will here be made of some of the evidence for what we may consider as "dynamic effects" of carcinogens in this sense. A consideration of the "energy changes in carcinogenesis" has been given by Rondoni (1955).

1. Metabolism and Excretion of Carcinogens

Perhaps the most clear-cut case where the specific carcinogenicity of a substance towards a particular organ can be ascribed to the liberation of a metabolite within the organ is the production of bladder cancer by 2-naphthylamine. This is dealt with in detail by Boyland (1958), but the essential dynamic process would appear to be the conversion of 2-naphthylamine to 2-amino-1-naphthol and conjugation with glucuronic acid by the normal detoxication process for phenolic substances. The glucuronide, however, when excreted in the urine, appears to be susceptible to the action of β-glucuronidase present in the bladder where the free deconjugated 2-amino-1-naphthol can act directly on the epithelial tissue, producing a cancer (Allen, Boyland, Dukes, Horning & Watson, 1957; Bonser, Bradshaw, Clayson & Jull, 1956).

The suggestion that excretion as glucuronide rather than as ethereal sulphate may have a special significance in determining carcinogenicity in aromatic amines has been put forward by Elson, Goulden & Warren (1958). In studying the excretion of aromatic amines they noted that although, with large doses of the amines in rats, increase in the urinary excretion of both ethereal sulphate (ester sulphate) and of glucuronic acid occurs, with lower doses there is a preferential excretion either as ethereal sulphate or as glucuronide.

In the limited series of aromatic amines examined, a sharp change in preferential conjugation of the amine from ethereal sulphate to glucuronide occurred on passing from the monocyclic aromatic amines (aniline, 4-chloroaniline (I), phenetidine (II), phenacetin (III), and acetanilide) to the more complex polycyclic amines (4-aminodiphenyl, benzidine, 4-aminostilbene (V) and 4-acetylaminostilbene). Whereas none of the amines excreted preferentially as ethereal sulphates has been found to be carcinogenic, all the amines excreted preferentially as glucuronides have now been shown to be carcinogenic in the rat (Walpole, Williams & Roberts, 1952; Spitz, Maguigan & Dobriner, 1950; Haddow, Harris, Kon & Roe, 1948). Walpole *et al.* (1952) have suggested that aromatic amines owe their carcinogenicity to metabolic conversion to the *ortho*-hydroxyamines (*ortho*-aminophenols) which are directly acting carcinogens, and Clayson (1953) has put forward a working hypothesis for the mode of carcinogenesis of aromatic amines based on this idea. It would be rash, however, to assume that metabolic hydroxylation in the position *ortho* to the amino group in an aromatic amine indicates that it will be carcinogenic, and in fact the drug



phenacetin (III), although most probably excreted as an *ortho*-aminophenol, showed no evidence of any carcinogenic action in very prolonged feeding tests in rats (Schmähl & Reiter, 1954). A similar case is represented by 4-chloroaniline (I) which has been shown by Elson and Spinks (unpublished) to be excreted in the rat as 5-chloro-2-aminophenol (IV), but prolonged feeding of 4-chloroaniline to rats failed to produce tumours.

Clearly other factors must also be involved in the carcinogenic potentialities of aromatic amines and it may be that preferential excretion as glucuronide is one of them. The significance of glucuronide excretion in the concept of dynamic aspects of carcinogenesis has been indicated in the suggestion (Elson, 1952) that

> . . . glucuronic acid formation and the role of glucuronidase in animal metabolism may not be exclusively concerned with the so called detoxification mechanisms, but may also be that of providing a transfer mechanism for conveyance of a fat-soluble but water-insoluble substance such as a steroid hormone from one organ in the body to others on which it is required to act. The substance is transferred by being converted into a water-soluble glucuronide, which may no longer show hormone activity, and this is carried in the blood stream, and, in this water-soluble form, is able to enter the cells of various organs. The active hormone is then liberated *in situ* in that organ by means of the glucuronidase present in the cell. On this conception the rather vague relation of glucuronidase to growth may be explained by the growth effects being really caused by the liberated hormone, and thus only indirectly related to the enzyme.

In discussing the hormonal regulation of enzyme activity Lardy (1957) lists the " physiological effects of oestrogens " as promoting uterine and mammary gland growth, together with the "enzyme responses *in vivo*" as increased glucuronidase in target organs, not in liver, kidney or spleen. The carcinogenic aromatic amines could thus become involved in this process in respect of their tendency to glucuronide formation, being transferred as their water-soluble glucuronides into the cells of the various organs, the active carcinogen being then liberated in situ by the glucuronidase present in the cells. It is interesting that Boyland, Wallace & Williams (1955) find that the urine of patients with cancer of the bladder usually contains abnormally high β-glucuronidase activity.

2. Growth-Inhibitory Action of Carcinogens

Twenty years ago Haddow, Scott & Scott (1937) found that certain carcinogenic hydrocarbons, particularly 1 : 2-5 : 6-dibenzanthracene, produced prolonged reduction of the growth-rate of young rats, whilst a number of chemically similar non-carcinogenic hydrocarbons did not show this effect. The carcinogens were also found to have considerable inhibitory effect on the growth of experimental animal tumours (Haddow, 1935; Haddow & Robinson, 1937).

Following a suggestion by White & White (1939, 1940) that the growth inhibition was the result of deprivation of essential sulphur-containing amino acids brought about by their combination with the carcinogen in the course of detoxication mechanisms involving the formation of mercapturic acids, Elson, Goulden & Warren (1945, 1947), in a study involving the urinary partition of sulphur in rats treated with carcinogenic hydrocarbons, concluded that, whilst the results supported the idea that combination with sulphydryl-containing substances is an early stage in metabolism of the carcinogens, their growth-inhibitory action is not a direct result of this reaction.

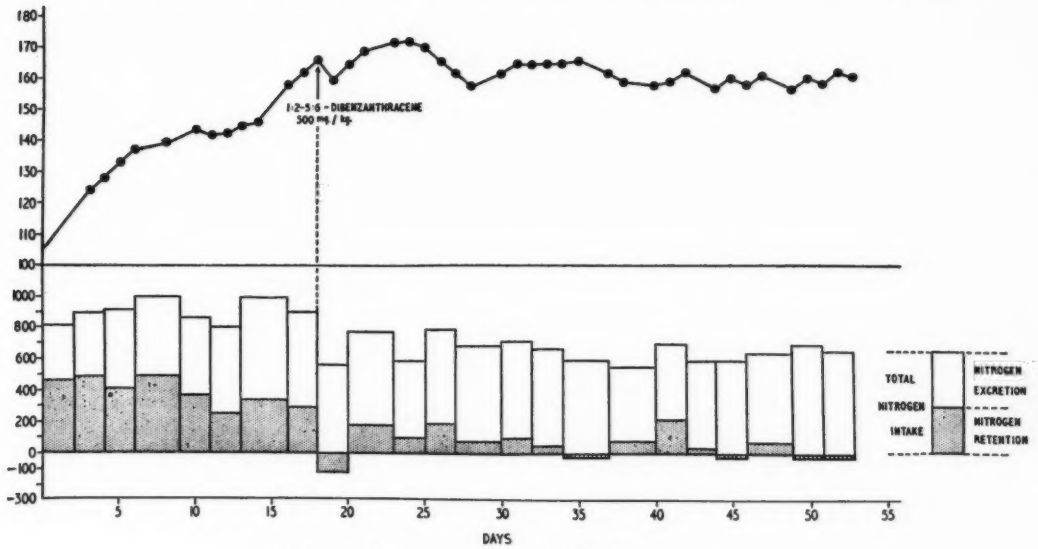
Subsequent work revealed a direct relation between the protein content of the diet of the animals and the growth-inhibitory action of carcinogenic substances (Elson & Warren, 1947; Elson, 1948, 1949) and it was suggested that the growth inhibition is caused by an interference with the availability or the actual synthesis of protein necessary for cellular growth. That the growth inhibition is not merely a general toxic effect resulting in the animal's decreased appetite, is shown by the fact that some rats react to treatment with 1 : 2-5 : 6-dibenzanthracene by maintaining or even increasing their daily food consumption although growth is almost completely inhibited (Elson, Kennaway & Tipler, 1949). In such animals therefore it would appear that a fairly profound change in basal metabolism has taken place. Figure 1 shows the result of a nitrogen balance determination in a rat treated with 1 : 2-5 : 6-dibenzanthracene. For the first day after treatment there is a slight loss in weight, and the animal shows a negative nitrogen balance. After a partial recovery the growth of the animal is completely inhibited and its weight remains practically constant for a prolonged period. During this period the total intake of food nitrogen is reduced by about 20%. The proportion of the nitrogen intake retained is, however, much less, whilst the amount of nitrogen excreted remains as great as, or even greater than, before treatment with the carcinogen. Moreover the major part of the nitrogen excreted is accounted for in the urine in the form of urea, faecal nitrogen being less than one-third of the total nitrogen excretion and remaining fairly constant before and after treatment. It would appear therefore that the effect of the carcinogen is to prevent the utilization of amino acids for protein synthesis and growth, whilst their utilization for energy metabolism is unaffected. Thus a young rapidly growing rat treated with the carcinogen shows an adult type of metabolism in that it utilizes more of its amino acids derived from its food protein for energy than it does for building up of tissue proteins for growth. In this respect the carcinogen can be considered to have an " ageing " effect on the animal. A similar effect has been observed during the short growth-inhibition period following whole body *x* irradiation (Elson and Lamerton, unpublished; White, Burr, Cool, David & Ally, 1955).

3. Enzyme Inhibition and Tissue Metabolism

Pursuing the idea that, during what should be the normal metabolic processes of detoxication, with some classes of carcinogens a metabolite is liberated in certain tissues, mention should now be made of some indications as to how such a harmful product could interfere with the metabolism of normal tissues, possibly imposing on them a more cancer-like type of metabolism.

The idea that uncontrolled growth of the cancer cell has its origin in some metabolic or enzymic abnormality has been developed mainly in relation to the energy derived from oxidative metabolism of fats and carbohydrate. The outstanding contribution was the discovery by Warburg (1930) of the high aerobic and anaerobic glycolysis of tumour tissue. Warburg (1956) now considers that cancer cells originate from normal cells in two phases, the first phase being injury to respiration, which can arise from any of the " . . . remote causes of cancer—tar, rays, arsenic, pressure, urethane . . ." The second phase, he suggests, is an adaptation phase in which in a prolonged struggle for existence some cells succeed in replacing irreversibly their lost respiration energy by

FIG. 1. NITROGEN BALANCE IN A RAT TREATED WITH 1:2-5:6-DIBENZANTHRACENE



Abscissae: days

Ordinates:
Upper: weight of rat (g.)
Lower: nitrogen (mg.)

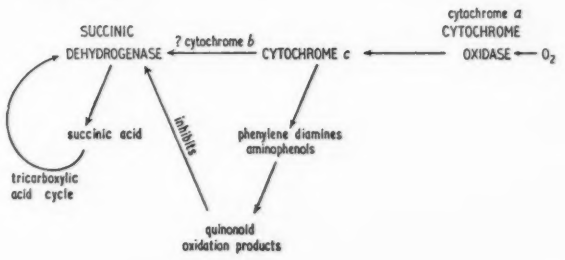
fermentation energy, and thus highly differentiated body-cells are converted into undifferentiated wildly growing cancer cells.

There is, however, much controversy over the question of tumour respiration, and Weinhouse (1955) concludes that "The high glycolysis of tumour tissue, whatever its cause, does not appear to be due to a radically altered respiratory metabolism." The evidence that high glycolysis is not an exclusive feature of neoplastic cells, as apparently all cells can glycolyse, particularly in response to damage (Rondoni, 1955), suggests that perhaps the glycolytic type of metabolism should now be considered as a consequence of malignancy, rather than a cause of it.

As an example of how the metabolites of certain carcinogens could cause biochemical damage to tissues, we may consider briefly the inhibition of enzymes by the metabolic products of carcinogenic azo compounds. The idea that metabolic products of carcinogenic azo compounds could inhibit enzymes was put forward by Kensler, Dexter & Rhoads (1942) who found inhibition of a coenzyme system from yeast by *p*-phenylenediamine (VII) and dimethyl-*p*-phenylenediamine, which had been shown to be metabolites of the carcinogenic 4-dimethylaminoazobenzene (VI). Potter (1942) and Potter & Dubois (1943) extended this work to the enzyme urease and to the succinoxidase system of rat liver. Elson & Hoch-Ligeti (1945) confirmed previous suggestions that an intermediate oxidation product of the aromatic amine metabolites, probably of a quinonoid structure, is responsible for their inhibitory properties and showed that this oxidation is brought about in the tissues by the cytochrome oxidase component of the succinoxidase system. Tissues of low oxidative capacity whose cytochrome oxidase is also low do not readily oxidase the amines, and their succinoxidase is not markedly inhibited. This lack of effect is observed on tissues such as spleen and testis which normally have a low oxygen uptake, and on tissues arising from organs such as liver which normally have a high oxygen uptake, but which have become impaired in their oxidative capacity by pathological changes. Thus the relatively low succinoxidase activity of tumours of the liver, and of tumours of other origin, was in general not inhibited by the amines.

It was later found that the degree of inhibition of liver succinoxidase is influenced by the diet of the animal. Maintenance on a diet deficient in vitamin B_1 leads to a decreased availability of cytochrome *c* for oxidation of the amine to the quinonoid inhibitory product, and consequently inhibition of the liver succinoxidase does not occur. Maintenance on a low-protein diet results predominantly in a decrease in the succinic dehydrogenase component of the system so that, since ample cytochrome oxidase and cytochrome *c* are available for oxidation of the amine, the already low dehydrogenase component of the system is very markedly inhibited (Elson, 1947). (See fig. 2.)

FIG. 2. INHIBITION OF SUCCINOXIDASE BY METABOLIC PRODUCTS OF CARCINOGENIC AZO COMPOUNDS



Preliminary work had suggested that a 5% protein diet, which results in considerable reduction of liver succinoxidase in rats, favoured the induction of liver tumours when 4-dimethylaminoazobenzene (VI) or 3′-methyl-4-dimethylaminoazobenzene was added to the diet. In a recent experiment (Doak & Elson, unpublished) it was found that albino rats maintained on this low (5%) protein diet to which 0.06% 4-dimethylaminoazobenzene had been added developed liver tumours in from 72 to 256 days. During this period only one tumour (at 248 days) was observed in rats fed on a high (20%) protein diet to which the same amount of the carcinogenic azo compound had been added. The majority of the liver tumours appeared in these rats in from 309 to 430 days and two animals were still free from tumours when killed 429 days after commencement of feeding of the carcinogen. The rats used in this experiment were from an albino colony which has a high growth-rate. When the experiment was repeated on August strain rats which grow much more slowly, even on the 20% protein diet, it was found that in this strain the high protein content of the diet conferred little protection against liver tumour induction by the 4-dimethylaminoazobenzene. Liver tumours appeared in the groups receiving high- and low-protein diets at approximately the same time as in the albino animals receiving the low-protein diet of the previous experiment.

It looks as though the high capacity for growth, and thus for protein synthesis and repair of damage particularly to enzyme systems, of the albino rats may be a major factor in protection against liver carcinogenesis. When we also take into consideration the growth-inhibiting potentialities of carcinogens in relation to protein synthesis (referred to in section 2) and to nucleic acid metabolism (Elson & Harris, 1947; Gopal-Ayengar, 1947), it would seem that more attention could profitably be paid in biochemical investigations of carcinogenesis to the links between protein metabolism and carbohydrate metabolism.

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EXPERIMENTAL LIVER TUMOURS

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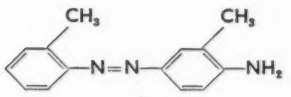
1 Experimental production and pathological features
2 Action of liver carcinogens: their possible relation to human liver cancer
3 Liver tumours and current concepts of carcinogenesis
References

Research on the cancer problem must include the study under experimental conditions of those lesions in animals that can be compared in some important respects with a naturally occurring malignant condition in man. Although comparatively rare in the United Kingdom or the USA, malignant liver tumours are an important cause of death among the comparatively young in many parts of the world. Since 1932, experimental malignant liver tumours in animals have been intensively studied. In the space allotted we cannot attempt a review of the literature on this subject, but we shall discuss briefly the relationship between the experimental findings and the human disease in order to see whether the study of one throws any light on the cause of the other. An excellent survey of the problem has been provided by Berman (1951) and, since his book appeared, two important conferences on liver tumours have been reported in full (Conference on Experimental Hepatomas, 1955; Conference on Carcinoma of the Liver, 1955).

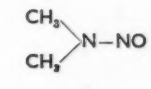
While no one doubts that malignant liver tumours are seen more frequently by pathologists in some areas than in others, adequate statistics are not always available for the different populations, and therefore the true incidence of the disease in relation to total mortality or to other forms of cancer cannot be assessed. There is already general agreement that neither race, diet nor the incidence of liver parasites accounts for the apparent liability of some populations to hepatoma. Nevertheless there seems good reason to accept the view expressed by Kennaway (1944) that some extrinsic causative factor must be sought. Although the incidence varies between peoples, the pathological picture does not; the lesions seen in the Bantu are indistinguishable from those observed in patients dying in Chicago (Popper, 1955). The incidence of liver cirrhosis may run parallel with a high incidence of tumours, but such an association may not always be found (Higginson, 1955). The incidence of cirrhosis probably reflects the frequency of liver disease as a whole in any particular population.

1. Experimental Production and Pathological Features

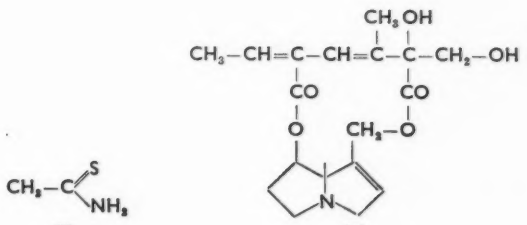
The first experimental hepatomata were produced by Yoshida (1932) in rats given *o*-aminoazotoluene (I). A great deal of work has been done on this and other azo compounds and aromatic amines, including attempts to correlate features of chemical structure with biological activity. Excellent reviews of this work are available (Badger & Lewis, 1952; Badger, 1956; Miller & Miller, 1953, 1955; Morris, 1955; Pullman & Pullman, 1955). Species differences in sensitivity to these compounds are observed but are no more striking than for those hydrocarbons producing skin tumours. More recently other compounds capable of producing malignant liver tumours have been described. Among the simplest chemical compounds are dimethylnitrosamine (II) (Magee & Barnes, 1956) and thioacetamide (III) (Fitzhugh & Nelson, 1948 ; Gupta, 1956). More complex ones include the pyrrolizidine (*Senecio*) alkaloids, the formula of one of which is illustrated (IV) (Cook, Duffy & Schoental, 1950; Schoental, Head & Peacock, 1954; Schoental & Head, 1955, 1957; Campbell, 1956). Other compounds have been described as producing liver tumours, but it is not always possible to state whether the lesion produced was a truly malignant tumour. Tannic acid is an interesting example of a substance capable of producing hepatomata after injection (Korpássy & Mosonyi, 1950) yet consumed in vast quantities without apparent harm by an enormous human population. This raises problems connected with the techniques for testing new chemicals for their carcinogenic action (Boyland, 1958).



I
o-Aminoazotoluene



II
Dimethylnitrosamine



III
Thioacetamide

IV
Riddelliine

In addition, nutritional disorders resulting from choline deficiency (Salmon, Copeland & Burns, 1955) or from feeding with ethionine may also produce liver tumours (Popper, de la Huerga & Yesinick, 1953; Farber, 1956). Clinically and pathologically the fully developed lesions produced in the rat by azo compounds, dimethylnitrosamine or pyrrolizidine alkaloids have the same features as those seen in man. There are gross changes in the size and shape of the liver, with a multiplicity of lesions distributed haphazardly throughout the organ and frequently accompanied by ascites, haemorrhages and both local and distant metastases. The histological picture is one of tremendous variety, particularly with respect to the proportion of hepatic to biliary tissue. Since the cells forming the biliary canaliculi are probably derived both embryologically and postnatally from hepatic cells (Horstmann, 1939), it is easy to understand why every possible variation in cell

composition may be found after a malignant transformation of hepatic cells has been induced. Cirrhosis is regularly found in rats on a choline-deficient diet but its onset is delayed if antibiotics are also administered (György, 1954; Rutenburg, Sonnenblick, Koven, Aprahamian, Reiner & Fine, 1957). Cirrhosis does not always follow the administration of the hepatic carcinogens. Tumours may appear in the absence of marked fibrosis and do not inevitably accompany a cirrhotic condition.

A more unusual and impressive change in livers damaged by the chemical carcinogens, in choline deficiency and after feeding with ethionine, is the appearance of very large hepatic cells showing many unusual cytological features (Orr, 1940; Stowell, 1949; Rather, 1951; Salmon *et al.* 1955; Popper *et al.* 1953; Bull, 1955; Schoental & Magee, 1957). Such cells are seen long before actual tumours appear. Bras (1955) has described similar polyploid cells in the livers of children recovering from kwashiorkor.

2. Action of Liver Carcinogens: Their Possible Relation to Human Liver Cancer

Most substances which produce liver tumours do not have a direct carcinogenic action when applied, for example, to the skin. These substances may be transformed into immediate carcinogens by metabolism. A great deal of work has been done on the metabolic transformation of the azo compounds, for example, and many metabolic products have been identified. However, none except the monomethyl derivatives has been shown to be carcinogenic. Recently it has been found that the methyl ethers prepared from the inactive hydroxy derivatives of dimethylaminoazobenzene are carcinogenic (Miller, Miller & Finger, 1957). Azo compounds are also bound to liver protein and it has been suggested that this might be a starting-point for tumour production (Miller & Miller, 1955). The production of similar malignant lesions by a compound like dimethylnitrosamine throws doubt on the significance of much of this work on metabolism. Dimethylnitrosamine will not bind to protein and it appears to be very rapidly metabolized (Magee, 1956; Dutton & Heath, 1956). Dimethylnitrosamine certainly interferes with protein synthesis carried out by the liver but it is uncertain whether this interference is the cause or an effect of its toxic action (Magee, 1957). If a metabolite is the immediate cause of liver cancer produced by dimethylnitrosamine, the compound must be basically a cellular component undergoing some change such, for example, as *O*-methylation (Kraychy & Gallagher, 1957) to which only a minor part of the original carcinogen has contributed. It is difficult to visualize the role of "chemical carcinogens" in a condition such as choline deficiency unless they arise from bacterial action in the gut.

Turning back to the human problem it is obvious that azo dyes, 2-acetamidofluorene, dimethylnitrosamine or thioacetamide can play no part in the induction of liver tumours in Africans or Asians. The case of pyrrolizidine alkaloids is somewhat different. They occur in many plants of the species *Crotalaria*, *Heliotropium* and *Senecio* which have been included in some herbal remedies throughout the world (Watt & Breyer-Brandwijk, 1932; Dalziel, 1937). Recent work on the toxic properties of the pyrrolizidine alkaloids (Schoental, 1957; Schoental & Magee, 1957) has shown that a single dose will produce a chronic persistent liver lesion in rats. When repeated doses are given, tumours may appear long after the administration has stopped (Schoental & Head, 1957). Liver damage is also produced in the young if these alkaloids are given to lactating rats (Schoental, unpublished observations). There seems, therefore, to be good reason for looking with suspicion upon herbal remedies made from plants containing these alkaloids and given to children and adults including nursing mothers. Chilli (*Capsicum frutescens* and *C. annuum*), a common ingredient of many diets in some parts of the world, is reported to have produced tumours in rats. The animals were on a partly synthetic diet containing ground-nut protein that produced fatty and cirrhotic livers in the controls. Of 26 rats receiving the diet with 10% chilli, 15 developed neoplastic changes in the liver (Hoch-Ligeti, 1951). No tumours were induced in mice and rats given chilli in normal diets (Hieger, 1940).

3. Liver Tumours and Current Concepts of Carcinogenesis

Can the production of liver tumours be fitted to current concepts of carcinogenesis? (See Berenblum, 1941.) For some tissues it has been possible to define a two-stage process of "initiation" followed by "promotion" (Friedewald & Rous, 1944).[1] This has not been shown conclusively in the case of experimental liver tumours. The initiating substances may be metabolites of the carcinogens themselves, with the reservation discussed above. Berenblum (1954) believes that the stage of promotion may be preceded by one of delayed maturation. This might readily occur in the liver during the regenerative processes which follow the cell damage produced in different degree by liver carcinogens. If regeneration is stimulated by partial hepatectomy in rats receiving butter yellow, the onset of tumours is hastened (Glinos, Bucher & Aub, 1951). Laws (1956) describes a similar phenomenon in rats receiving 2-acetamidofluorene.

The nature of an initiating agent cannot be surmised. On a chemical basis it is difficult to visualize a common one produced by all the known liver carcinogens. On the other hand it might be more profitable to study further the conditions affecting promotion. For example, in the United Kingdom liver carcinoma is not seen in the cirrhosis which follows the single insult of a viral hepatitis, but only in the cirrhosis of the alcoholic where the liver has been repeatedly injured (Parker, 1957). The role of diet should not be overlooked (Tannenbaum & Silverstone, 1953). Poor diets raise the incidence of tumours in rats given azo compounds; riboflavine reduces the numbers of tumours. In the case of the pyrrolizidine alkaloids a protein-rich diet appears to favour the development of tumours (Schoental & Head, 1957). A single dose of a pyrrolizidine alkaloid produces a galaxy of bizarre cells persisting for periods up to two years, but no tumours have so far appeared unless the dose is repeated. The possibility that repeated liver damage by another—non-carcinogenic—agent would induce tumours is being investigated.

Just as studies of skin tumours in animals may help in understanding the origin of the human lesion due to cutting oils, so may further work on experimental hepatomata in animals throw some light on factors responsible for one of the most serious malignant conditions in many parts of the world. Much more information is needed on the dietary and other environmental factors at work among the groups with a high incidence of this condition.

[1] See also Salaman, p. 116 of this number of the Bulletin.—Ed.

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NUCLEAR AND ALLIED RADIATIONS AND THE INCIDENCE OF LEUKAEMIA IN MAN

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1 Incidence of leukaemia after irradiation
  a Leukaemia among survivors of atomic explosions
  b Leukaemia among patients irradiated for diagnostic or therapeutic purposes
  c Leukaemia among radiologists
  d Conclusions
2 The relationship between the incidence of leukaemia and the radiation dose
  a Available data
  b Implications of a linear dose-response relationship
References

In nearly every country death-rates have fallen sharply in the last 20 years. For some few diseases, however, death-rates have risen and, among them, leukaemia is one of the most outstanding. In England and Wales the increase in the leukaemia death-rate has been substantial (from 17 per million living in 1931 to 50 per million in 1955), and the increase has been proportionately greater than for any cause of death other than cancer of the lung and coronary thrombosis (Hewitt, 1955). Data for six countries from 1940 to 1955 are shown in Table I.

To some extent, the increase may be due to changes in diagnostic methods and fashions in medical terminology, but there are good reasons for believing that at least some of the increase reflects a real rise in the incidence of the disease. During this same period the use (and abuse) of ionizing radiations has increased extensively, and it is, therefore, pertinent to examine the data that have led to the belief that radiations can cause leukaemia in man and to try to determine the quantitative relationship between the degree of exposure to radiation and any subsequent increase in the incidence of the disease.

TABLE I.* DEATH-RATES FROM LEUKAEMIA PER MILLION PERSONS IN VARIOUS COUNTRIES, 1940–55

Year	Canada	Denmark	Eire	England and Wales	Scotland	USA
1940	30	48	15	26	38	39
1941	31	44	16	25	28	39
1942	31	39	16	28	27	40
1943	30	52	24	30	32	41
1944	36	51	22	31	32	43
1945	33	57	16	34	32	45
1946	36	52	22	35	34	47
1947	37	57	17	35	32	51
1948	39	58	24	35	30	53
1949	45	61	20	40	39	55
1950	46	59	26	42	39	59
1951	46	62	32	44	43	61
1952	46	64	32	46	44	63
1953	53	69	36	48	38	63
1954	51	70	—	49	48	65
1955	—	72	—	50	48	—

* Based on Tables 1 and 2, in Court-Brown & Doll (1957).

1. Incidence of Leukaemia after Irradiation

a. Leukaemia among Survivors of Atomic Explosions

It is estimated by Oughterson & Warren (1956) that about 255,000 people in Hiroshima and at least 173,000 people in Nagasaki were exposed to the atomic explosions over the cities in 1945. The first published account to provide evidence of an increase in the incidence of leukaemia amongst the survivors was that of Folley, Borges & Yamawaki (1952). Since then other accounts have appeared, notably those of Lange, Moloney & Yamawaki (1954), Yamawaki (1954), Moloney & Kastenbaum (1955) and Moloney (1955).

From data supplied by the Atomic Bomb Casualty Commission to the Medical Research Council's Committee on the Hazards to Man of Nuclear and Allied Radiations it is known that, by the end of August 1955 among the Hiroshima survivors (some of whom were then no longer resident in the city), no fewer than 125 cases of leukaemia had been confirmed, and in 23 other cases the diagnosis was suspected or under review. In the corresponding period in Nagasaki 40 cases had been confirmed and in 16 others the diagnosis of leukaemia was thought probable. The sex and age distribution of the survivors are known in greater detail for Hiroshima than for Nagasaki, and Court-Brown & Doll (1956) have used the Hiroshima data to estimate the extent of the increase in the incidence of leukaemia. They found that by the end of 1954 there had been nearly a fivefold increase in the number of cases occurring among the survivors still resident in the city, in comparison with the number that might have been expected from the general mortality data for Japan. Furthermore, the ratio of the number of cases observed to the number expected decreased progressively the further away the survivors were from the hypocentre[1] at the time of the explosion (Table II). The incidence of the disease among the survivors in both cities is shown in Table III for each of the years from 1946 to 1954. Each case is ascribed to the year in which clinical symptoms developed and, as the diagnosis may not be established for one or more years after the onset of symptoms, it must be concluded that there is no convincing evidence of a diminishing incidence, even by the end of 1954.

Examination of the different types of leukaemia shows that the increase has been confined to the acute types of leukaemia and to chronic myeloid leukaemia. Only five cases of chronic lymphatic leukaemia had been reported by the end of August 1955. This may be held to support the argument that ionizing radiations do not increase the incidence of chronic lymphatic leukaemia, but the situation is complicated by the fact that chronic lymphatic leukaemia is uncommon in all parts of Japan. More convincing evidence of the lack of increase in the incidence of chronic lymphatic leukaemia after irradiation has been obtained from studies of Western populations (see below).

[1] An imaginary point on the ground directly beneath a nuclear device exploded at some distance above ground level.

TABLE II.* INCIDENCE OF LEUKAEMIA AMONG SURVIVORS OF THE HIROSHIMA ATOMIC BOMB EXPLOSION: PERSONS SUBSEQUENTLY RESIDENT IN HIROSHIMA CITY ONLY

Distance from hypocentre at time of explosion (m.)	Number of survivors on 1 October 1950†	Number of cases of leukaemia		Incidence per 10,000 persons (total cases)
		Confirmed	Suspected	
Less than 1,000	1,250	16	0	128.0
1,000–1,499	10,350	28	1	28.0
1,500–1,999	18,450	6	1	3.8
2,000–2,999	30,350	7	0	2.3
3,000 or more	37,700	4	2	1.6
All distances	98,100	61	4	6.6

* Taken from Table 1A, in Court-Brown & Doll (1956).
† The numbers of survivors have been rounded off to the nearest 50.

The estimates differ slightly from those published by Moloney & Kastenbaum (1955) in accordance with data provided by the Atomic Bomb Casualty Commission.

b. Leukaemia among Patients Irradiated for Diagnostic or Therapeutic Purposes

A number of reports have appeared of patients developing leukaemia after treatment with *x* rays, radium or radioactive isotopes or after the diagnostic use of radioactive iodine (Merklen, Wolf & Néel, 1925; Engelbreth-Holm, 1941; Gerbis, 1943; Lynch, 1951; Delarue, Tubiana & Dutreix, 1953; Seidlin, Yalow & Siegel, 1954; Seidlin, Siegel, Melamed & Yalow, 1955; Blom, Querido & Leeksma, 1955; Pochin, Myant & Corbett, 1956; Abbatt, Farran & Greene, 1956).

In addition, there have been two surveys of the incidence of leukaemia after radiotherapy and two studies of the extent to which patients with leukaemia have been previously treated with *x* rays or been previously examined radiographically.

TABLE III.* INCIDENCE OF LEUKAEMIA IN DIFFERENT YEARS AMONG SURVIVORS OF THE HIROSHIMA AND NAGASAKI ATOMIC BOMB EXPLOSIONS: PERSONS SUBSEQUENTLY RESIDENT IN HIROSHIMA CITY AND NAGASAKI ONLY

Year of onset	Hiroshima (confirmed and suspected cases)	Nagasaki (confirmed and suspected cases)	Incidence per 10,000 persons: Hiroshima and Nagasaki
1946	0	1	0.05
1947	3	2	0.3
1948	10	3	0.7
1949	5	3	0.4
1950	7	6	0.7
1951	11	10	1.1
1952	11	8	1.0
1953	10	3	0.7
1954	6	7	0.7
All years	63†	43	5.6

* Based on Table 3A, in Court-Brown & Doll (1956).
† Two more cases are known in Hiroshima, one in which the year of onset is not known, and one occurring in the first part of 1955.

In 1955, Simpson, Hempelmann & Fuller reported the results of a survey of 1,722 children who had been irradiated during infancy for enlargement or suspected enlargement of the thymus gland at one or other of five centres in the United States of America. A control series consisted of 1,795 sibs of the treated children. Of the treated group 1,400 were traced and 17 were found to have developed cancer, including 7 who had developed leukaemia. In the control group only 5 children had developed cancer and none had leukaemia. Calculations based on incidence rates in New York State showed that less than one case of leukaemia would have been expected in either group. In a more recent report Simpson & Hempelmann (1957) draw attention to the difficulty of assessing this evidence; for it is not known to what extent the thymi of the control children differed from those of the treated children, and the possibility cannot be excluded that an enlarged thymus during infancy may be associated with a heightened tendency for the development of leukaemia in childhood. In fact it is conceivable that the condition may have been present at the time of irradiation in some of the children who subsequently manifested the disease.

That the incidence of leukaemia among patients treated with *x* rays for ankylosing spondylitis appeared to be unusually high was reported independently by Court-Brown & Abbatt (1955) and by van Swaay (1955). A large survey of such patients was, therefore, carried out on behalf of the Medical Research Council's Committee on the Hazards to Man of Nuclear and Allied Radiations, and the full results were published by Court-Brown & Doll (1957). In this survey the case records were examined of 13,352 patients, presumed to have had ankylosing spondylitis and who had been treated at 81 British radiotherapy centres between 1 January 1935 and 31 December 1954. A diagnosis of leukaemia, aplastic or hypoplastic anaemia, or myelofibrosis was recorded on the death-certificates of 46 of the patients who had died by December 1955, and 3 further patients known to be suffering from leukaemia were still alive. In 28 of the 46 patients, death was certified as attributable to leukaemia; whereas the expected number, calculated from the national vital statistics, was 2.9. This calculation was made on the assumption that the probability of developing leukaemia among subjects of spondylitis who had not been irradiated was the same as for members of the general population of the same age and sex. Some support for this assumption came from a survey carried out by Abbatt & Lea (1956) who found a substantial increase in the incidence of leukaemia among 1,627 men discharged from the armed services and awarded pensions for ankylosing spondylitis, who had had *x*-ray treatment. In contrast, no case of leukaemia was found among 399 pensioned men with spondylitis who had not had *x*-ray treatment. The number of untreated men in this survey is, however, small and the possibility that there may be some slight association between spondylitis and leukaemia cannot be completely excluded. Nor can the possibility be excluded that the spondylitic may be more susceptible than normal to the induction of leukaemia by irradiation, by virtue of some abnormality of his haemopoietic system. The increase in leukaemia observed in the Medical Research Council's study was due to an increase in the chronic myeloid and the acute forms of the disease, and no evidence was found of an increase in chronic lymphatic leukaemia. The latent period in those cases of leukaemia that developed after a single course of *x*-ray treatment was most commonly between three and five years.

A study of the aetiology of malignant disease in childhood, with special reference to the significance of irradiation in utero in the production of leukaemia, has been carried out at the Department of Social Medicine, Oxford. A preliminary report (Stewart, Webb, Giles & Hewitt, 1956) drew attention to the finding that the mothers of 42 out of the 269 children with leukaemia said that they had had abdominal *x*-ray examinations during the relevant pregnancies, whereas the mothers of only 24 out of the 269 control children gave a similar history. No difference was found between the two groups with regard to *x*-ray exposure of the mothers prior to conception of the child or with regard to postnatal *x*-ray exposure of the children.

In Denmark, Faber (1957a) has studied 828 cases of leukaemia notified to the Danish Cancer Registry during the years 1950 to 1953. In view of the difficulty of collecting evidence of diagnostic *x*-ray exposure received many years before admission to hospital, his most important data are, perhaps, those relating to a history of diagnostic exposure within five years of the clinical development of the disease. He found that 8.1% of 283 patients with chronic lymphatic leukaemia gave a history of exposure within five years of the onset of their disease. Among 150 patients with chronic myeloid leukaemia the comparable incidence was 11.8%, and among 395 patients with acute leukaemia it was 17.3%. When diagnostic exposure more than five years before the development of leukaemia was considered, the incidence was 8.4% for chronic lymphatic leukaemia, 12.6% for chronic myeloid leukaemia and 8.7% for the acute leukaemias. The incidence of previous therapeutic irradiation differed more markedly between the groups; in chronic lymphatic leukaemia it was 1.8%, in chronic myeloid leukaemia it was 7.8% and in the acute leukaemias it was 8.0%. Faber assumed that the incidence of chronic lymphatic leukaemia was not increased by irradiation and, therefore, used the frequency of a history of *x*-ray exposure in this condition as a control. On this basis, he argued that both the acute leukaemias and chronic myeloid leukaemia can be induced by irradiation, that the latent periods in the former are five years or less, but that in the latter the latent periods might be longer. He further concluded that some cases in his series must have been caused by diagnostic *x*-ray exposure. In a more recent communication he has reported that another survey has shown that the incidence of *x*-ray exposure in control patients with diseases other than leukaemia does not, in fact, differ significantly from that in patients with chronic lymphatic leukaemia (Faber, 1957b).

c. Leukaemia among Radiologists

In 1942 Dunlap drew attention to 24 cases of leukaemia reported in the literature as occurring among radiologists and associated workers. Since then a number of reports suggested that the mortality from leukaemia among American radiologists is unusually high. March (1944, 1947, 1950) and Ulrich (1946) analysed the causes of death of physicians recorded in the obituary notices of the *Journal of the American Medical Association*. March found that, for the period 1928–1948, 334 deaths were reported as due to leukaemia among a total of 65,922 reported deaths of physicians not practising radiology (i.e., 0.5%). In contrast, among the deaths of 299 radiologists, 14 were due to leukaemia (4.7%). From a comparison of these two proportions it has been suggested that radiologists suffer an approximately ninefold increase in their death-rate from leukaemia. The cause of death, however, was not given in 20–25% of the obituary notices, and it is possible that, in view of its special interest, leukaemia might be recorded more readily as a cause of death in a radiologist than in a physician not practising radiology. Moreover, no data are given to indicate that the two populations are directly comparable with regard to their age distribution. In the light of these objections, and even though the data published by these two authors almost certainly point to an increased liability to leukaemia among radiologists, it appears unjustifiable to conclude that this liability has been as much as nine times greater than that of other physicians.

Other data have been published by Dublin & Spiegelman (1947, 1948). These authors studied the records and causes of death of physicians in the American Medical Association during the period 1938 to 1942. They found that the mortality among male physicians from leukaemia was 175% of that among white males in the general population with a comparable age distribution and that this excess was greater than that for any other disease group. The group of 175,159 physicians could, moreover, be divided into non-specialists and specialists, and the latter could be subdivided into different categories of specialization. Dublin and Spiegelman were, therefore, able to calculate mortality ratios for various diseases and groups of diseases for each specialty; that is, they calculated the ratio between the numbers of observed deaths and the numbers of deaths expected if the subgroup under consideration had been subject to the mortality rates of the whole group of physicians. For cancer as a whole the mortality ratio among radiologists was 1.33 to 1; although radiologists constituted only 4.3% of the specialists, they accounted for one-quarter of all the deaths from leukaemia occurring among them. This finding was regarded by Dublin and Spiegelman as most likely due to a special occupational hazard.

d. Conclusions

The findings of the Atomic Bomb Casualty Commission, the results of surveys on the incidence of leukaemia in irradiated patients, and the increased leukaemia mortality recorded among radiologists, all suggest that ionizing radiations can cause leukaemia in man. Difficulties in interpretation have arisen in some of these investigations, but if all are taken together the data provide convincing evidence of man's susceptibility to the induction of leukaemia by irradiation. It now seems established that radiations do not increase the incidence of chronic lymphatic leukaemia but that they increase the incidence of the acute leukaemias and of chronic myeloid leukaemia. There is some conflict of evidence, in regard to the average length of the latent period, between the Japanese data and the findings of both the spondylitic and Danish surveys. It seems reasonable, however, to conclude that in Western Europe the latent period before the appearance of the acute types of leukaemia is usually from three to five years, but that it may well be longer in some cases of chronic myeloid leukaemia.

2. The Relationship between the Incidence of Leukaemia and the Radiation Dose

If it is accepted that ionizing radiations are leukaemogenic for man, it then becomes important to investigate the nature of the relationship between the dose of radiation and the

resulting incidence of the disease, and particularly to determine whether there is any evidence of a threshold dose below which no increase in the incidence of the disease will be produced. The problem is difficult because if it is studied retrospectively the investigator is beset by the lack of data on the doses received by patients or, where they are recorded (as in the cases of spondylitis), by the absence of any data on patients treated with low doses. A prospective study, on the other hand, is difficult to organize because of the large number of patients who have to be followed to obtain a reliable estimate of the incidence.

a. Available Data

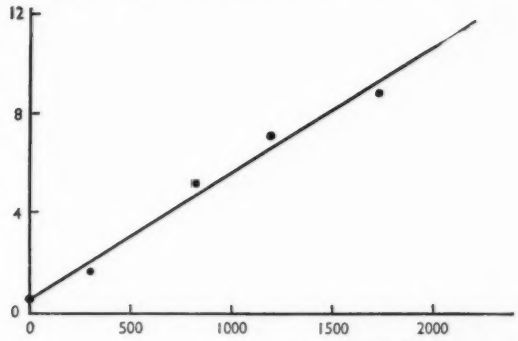
The data of Simpson and her co-workers suggest that, in children who have had thymic irradiation, leukaemia may be produced by doses of 200 r. or less restricted to the thorax, and the work of Stewart and her collaborators suggests that doses of not more than 5 r. to the foetus may result in the subsequent development of leukaemia in postnatal life. But neither survey provides data which indicate the nature of the dose-response relationship. That there is such a relationship is indicated by the progressive manner in which the incidence of leukaemia among the survivors of Hiroshima decreases the further they were situated from the hypocentre at the time of explosion (Table II). Three difficulties, however, prevent the construction of a formal dose-response relationship from the Japanese data. Firstly, although estimates have been published by the Los Alamos Scientific Laboratory (1950) of the immediate γ-ray dose received at varying distances from the bomb, reliable data are lacking on the γ-ray dose contributed by subsequent fall-out. Secondly, it has not been possible to make reliable estimates of the dose due to neutron emission, as this requires a knowledge of the energy spectrum of the emitted neutrons; which in turn requires a detailed knowledge of the geometry of the bomb components at a complex stage of their dispersal. Even if the neutron dose were known, assumptions would have to be made about the relative biological efficiency, for the induction of leukaemia, of neutrons of differing energies. Thirdly, even if it were possible to estimate accurately the dose of radiation, it would be necessary to allow for the different manner of irradiation of the inhabitants of Hiroshima and Nagasaki—which was by no means uniform. Some received whole body irradiation but many must have received varying degrees of partial body irradiation, depending upon whether they were indoors or out-of-doors and whether they were partially shielded by adjacent masonry.

The estimation of the relevant dose to the bone-marrow in the survey of leukaemia among the spondylitis patients was also difficult but it presented fewer problems, and an attempt was made to derive a quantitative relationship between the observed incidence of leukaemia and the radiation dose (Court-Brown & Doll, 1957). For this purpose a sample of 2,254 patients was selected (approximately one in six of the whole series) and details of the *x*-ray treatment given were abstracted from their case records. Since it appeared that there might be a difference in sex susceptibility to the induction of leukaemia, the estimate of the dose-response relationship was based on the data obtained for male patients only. Three different parameters of dose were studied, of which the most useful was thought to be the mean dose in röntgens to the whole spinal bone-marrow. This dose was measured in a full-scale model of the human trunk into which were incorporated a skeleton and artificial lungs. In ankylosing spondylitis the major site of involvement is the spinal column, but extra-spinal joints such as those of the hip and shoulder may also become involved and consequently be treated with *x* rays. The dose to the extra-spinal marrow resulting from the treatment of these areas could not be estimated and consequently the most accurate form of the dose-response relationship was that based on data for patients with irradiation of the spinal column only. Of the 61,902 man-years[2] at risk following exposure of the whole group of spondylitics to therapeutic *x* rays, no fewer than 40,247 were man-years following spinal irradiation alone. Eighteen cases of leukaemia were found in this subgroup and the result of relating the annual incidence of leukaemia per 10,000 men to the mean dose to the spinal marrow is shown in fig. 1.

Support for the fact that the relationship may be linear, at least over the lower levels of dose, is gained by a study of the dose-response relationship for the whole group of male patients; that is, by including those who had had extra-spinal irradiation and who had received, in many cases, an important contribution to their spinal dose from this source. Altogether 37 cases of leukaemia were available for study and, while the best-fitting curve is concave upwards (fig. 2), the lower five points may well lie on a straight line. In both forms of the relationship the annual incidence for zero therapeutic dose was calculated to be 0.49 cases per 10,000 men. This figure was calculated from the mortality rates in Britain for the relevant years and was adjusted to include cases comparable

[2] Man-years indicate the product of multiplying the number of persons in a population by the number of years for which they have been observed. When this product is in its turn multiplied by the appropriate mortality rate, the answer derived is the number of deaths expected in the relevant population from the cause being investigated.

FIG. 1. THE INCIDENCE OF LEUKAEMIA, STANDARDIZED FOR AGE, IN RELATION TO THE MEAN DOSE OF RADIATION TO THE SPINAL MARROW; PATIENTS GIVEN EXTRA-SPINAL IRRADIATION HAVE BEEN EXCLUDED



Court-Brown & Doll (1957) by permission of HMSO

Abscissae: mean dose to spinal marrow (r.)

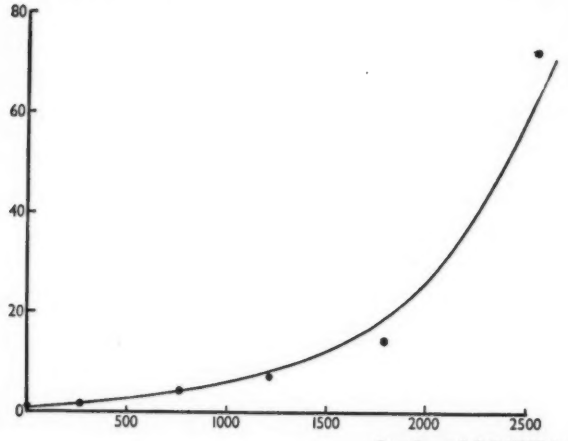
Ordinates: annual incidence of leukaemia per 10,000 men

$$y = 0.00520x + 0.49$$

$$(y = 0;\ x = -94)$$

Some variation in the position of the points occurs, depending on the particular subgroups of dose chosen. For details of this effect, see Court-Brown & Doll (1957).

FIG. 2. THE INCIDENCE OF LEUKAEMIA, STANDARDIZED FOR AGE, IN RELATION TO THE MEAN DOSE OF RADIATION TO THE SPINAL MARROW; INCLUDING PATIENTS GIVEN EXTRA-SPINAL IRRADIATION



Court-Brown & Doll (1957) by permission of HMSO

Abscissae: mean dose to spinal marrow (r.)
Ordinates: annual incidence of leukaemia per 10,000 men

to those included in the survey irrespective of whether they were dead or alive.

Two important assumptions had to be made in the construction of these relationships. Firstly, to calculate the number of years for which the patients had been exposed to risk it was assumed that leukaemia was equally likely to develop at any year following irradiation with the exception of the first year, when it was assumed that the time for its development was too short for any cases to become clinically manifest. This postulate is at variance with the clinical finding that the commonest time for the development of leukaemia following a single course of *x*-ray treatment is between the third and fifth year. At the time at which the study was undertaken it was thought that greater weight should be given to the evidence on the length of the latent period obtained from the more extensive Japanese data. A dose-response relationship was, however, also derived on the assumption that the maximum probability of developing leukaemia occurred in the fourth and fifth years after exposure with decreasing probabilities in the earlier and later years. The resulting relationship between the incidence of leukaemia and dose was less regular, but showed no evidence of a threshold dose below which no increase in incidence occurred, nor any evidence that at higher dose levels the incidence increased more rapidly than would be expected from a linear relationship.

Secondly, an assumption had to be made about the significance of repeated courses of treatment; 46% of all the patients received more than one course and among these were 27 cases of leukaemia. In the absence of any information to the contrary it was assumed that the probability of development of leukaemia was determined only by the total amount of radiation, irrespective of the number of courses given and the way in which the dose was fractionated. The spinal marrow doses from each course were, therefore, simply added together. This assumption is most probably an over-simplification. For example, if a patient has two identical courses of treatment to the same anatomical area, it is quite probable, as is suggested by the findings of Stewart & Dische (1956), that the number of bone-marrow cells at risk to the second treatment will be less than during the first, with a consequent decrease in the probability of the induction of leukaemia. Also *x*-ray treatment given subsequent to the establishment of the leukaemic process but prior to its becoming clinically manifest must be wasted radiation, as far as leukaemogenesis is concerned, and should not enter into the relationship between dose and incidence.

From the data summarized in fig. 1 it can be calculated that the dose to the marrow which would be expected to double the incidence of leukaemia is approximately 94 r. If it is assumed that the relationship is linear over low doses of radiation, and that between one-third and one-half of the total body red marrow is contained in the spinal column, it can be calculated that the dose to the whole body red marrow which would double the leukaemia incidence lies between 30 r. and 50 r. In other words the probability of developing leukaemia per year lies between 1×10^{-6} and 2×10^{-6} per r. averaged over the whole red-marrow system. For example, if an individual received an average dose of 10 r. to the whole red-marrow system, then he would incur an annual risk of developing leukaemia of between 10 and 20 chances in a million. Lewis (1957), after reviewing all the other available data on radiation-induced leukaemia in man, has come to a similar conclusion, and his findings support the opinion expressed by Court-Brown and Doll that the hypothesis of the induction of leukaemia in man by ionizing radiations as a non-threshold effect is reasonable in the light of the known facts. However, this is not the only interpretation possible, and, while it is advanced as a useful working hypothesis, it should be borne in mind that a number of authorities disagree with this view.

b. Implications of a Linear Dose-Response Relationship

If no threshold dose exists for the induction of leukaemia, it follows that a proportion of cases of leukaemia will result from natural background radiations and that it would be reasonable to suppose that the induction of the disease depends on the production of a somatic mutation in a precursor cell. It is believed that natural background radiation is responsible for a certain proportion of naturally occurring germinal mutations, and Muller (1954) has drawn attention to the frequency with which agents that produce germinal mutations are also carcinogens. In England and Wales the estimated annual dose to the gonads from natural background radiation, which may be used as a guide to the dose which the bone-marrow receives, is about 0.1 r., but this level must vary from locality to locality, depending mainly on variations in the amount of terrestrial radiation. Lewis (1957) used the data of MacMahon & Clark (1956) on the incidence of leukaemia in the borough of Brooklyn, New York, to estimate the proportion of the cases of leukaemia which might be due to background radiation, and he concluded that with a dose-rate of 0.1 r. per year the proportion of cases which might be attributable to background radiation was about 10%. It is true in theory that for a non-threshold effect the yield should be constant for a given dose irrespective of the dose-rate, but in practice, however, it may well be that some cells

irradiated at a high dose-rate will die which, exposed to a low dose-rate, would have survived to carry the initial change responsible for the leukaemic process. Deductions made from the effects of therapeutic and diagnostic irradiation to the effects of natural background radiation may therefore be misleading.

In the light of our present knowledge it is not possible to make reliable quantitative estimates of the effect of an increasing background exposure from artificial sources, but there is little doubt that an increased exposure is taking place and, if the induction of leukaemia is a non-threshold effect, it will result in the occurrence of more cases of leukaemia. By far the most important cause of this increase is the use of *x* rays in diagnostic radiology. Estimates made in England and Wales and in other countries suggest that at present the gonad dose to the population, from diagnostic procedures, may be from about 20% to nearly 100% of the dose from natural background radiation, and there are many indications that the number of diagnostic *x*-ray exposures is increasing each year. If the basic hypothesis of the non-threshold nature of leukaemia induction is correct, some cases of leukaemia must have resulted from the use of *x* rays in diagnosis; but it is unlikely that this source alone can account for the whole of the increase that has occurred in the national death-rate from leukaemia.

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THE DEVELOPMENT OF LEUKAEMIA IN IRRADIATED ANIMALS

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1 Murine leukaemia
2 Radiation-induced leukaemia
3 The role of the thymus
4 Dose-rate and the leukaemogenic mechanism
References

The induction of leukaemia in man by penetrating radiation is now a well-established fact (Court-Brown, 1958). The major problems at the moment are the mechanism by which radiation produces leukaemia and the existence or not of a threshold dose below which leukaemia will not be produced. The two problems are closely connected, since the leukaemogenic mechanism must determine the relation between dose of radiation and incidence of leukaemia. In fact the only evidence about the leukaemogenic mechanism in man which is available at present is the dose-response relation, which has been determined very approximately in the Japanese who received a single, very short exposure of radiation from the explosion of atomic bombs, and rather more accurately in the British patients with ankylosing spondylitis who each received one or more courses of treatment consisting of repeated exposures to x rays (Court-Brown & Doll, 1957). Animal experiments can clearly be designed to cover a much wider range of conditions of exposure to radiation than human experience can offer and should be capable of providing critical tests of hypotheses in so far as dose-response relations can give information about mechanisms. Animal experiments, too, provide opportunities for experimental interference of a kind impossible in man. The value of animal experiments, however, may depend on how closely the animal disease mimics the human (cf. Israëls, 1954; Kaplan, 1954).

1. Murine Leukaemia

Leukaemia has been found to occur naturally in a variety of mammals but has been regularly produced by experimental irradiation only in mice. (The occurrence of leukaemia in chronically irradiated guinea-pigs has been reported only once (Lorenz & Congdon, 1955).) The kind of leukaemia most commonly found in irradiated mice is the mediastinal type, as reported in the first extensive account of experimental carcinogenesis by whole body irradiation of mice (Furth & Furth, 1936). Here the thymus may appear to the unaided eye to be the only organ involved; the superior mediastinum is occupied by a soft tumour mass which may take up as much space as all the rest of the contents of the chest put together. The clinical picture is one of dyspnoea resulting from mechanical interference with breathing and is thus totally unlike that of any human case of leukaemia.

At the other extreme there is generalized enlargement of the lymph nodes—including the paratracheal lymph nodes—and of the thymus, with infiltration of liver, spleen and bone-marrow. Thymic involvement, however, may not be detectable by the unaided eye and the clinical picture is then closely similar to some cases of human leukaemia. All grades of thymic involvement and lymph-node non-involvement may be found, and whether murine lymphoid leukosis is thought to resemble or to differ from human leukaemia depends very much on which of the two extreme pictures the observer considers most typical of the disease in the mouse. The physiological involution of the thymus with increasing physiological age is much delayed in the mouse as compared with man and, if the thymus is regarded as a normal lymphoid organ (Trowell, 1958), its involvement in murine lymphoid leukaemia might be considered natural. Myeloid leukaemia is less frequently induced by irradiation of mice just as its spontaneous occurrence is rarer than that of lymphoid leukaemia; the opposite is true in man.

An abnormally high peripheral white blood-cell count is more characteristic of human leukaemia than of mouse leukaemia but it is now generally recognized that tissue involvement, as in the bone-marrow, is a more fundamental feature. It must be recognized, however, that leukaemia is an unsuitable name for the murine disease.

Besides typical myeloid and lymphoid leukaemia many other types of proliferative disease of reticular tissue can be defined in man and in the mouse. Dunn (1954) has attempted a comprehensive histological classification into which may be fitted many of the murine cases. My experience confirms hers that the kinds of disease differ in their relative frequency in genetically different mouse stocks, and also that the difficulty of classification, the indefinite merging of one type into another through a series of examples each differing only slightly from the next, varies greatly between genetically different mouse stocks. When it is important to be able to classify reasonably well each case of leukaemic disease which may occur, as must be done when determining dose-response curves to irradiation, the choice of the mouse strain to use for the experiments is of great moment. A strain with a high spontaneous incidence of some kind of leukaemia may well reduce the difficulties of classification, but this advantage is more than counterbalanced by the disadvantage of the short natural life-span and the fact that the difficulty of establishing with statistical certainty the reality of a small increase in incidence when the control incidence is of the order of 50% is greater than when it is nearly zero.

In this laboratory the CBA mouse has been used for much of the work on mammalian radiation damage. The strain was originally developed by selection for longevity so that there is a low incidence of killing disease during the first two years of life. The mean age at death in our laboratory is about 27 months for males, 29 months for females. Thus, if a mouse is exposed to radiation for a period as long as several months, there is still plenty of time for the delayed effects of radiation to manifest themselves before mortality in the controls begins to complicate the interpretation of the results.

In the CBA mouse the distinction between the different forms of murine leukaemia seems to be clearer than in the other strains used (see Table I). The clinically distinct types of lymphoid leukaemia are the mediastinal and the generalized types already described, and a third type where spreading soft subcutaneous masses are found commonly in the axillae, on

TABLE I. INCIDENCE OF LEUKAEMIA IN VARIOUS MOUSE STRAINS AFTER 6 OR 4 WEEKS *x* IRRADIATION (50 r. DAILY)

Strain	Sex	Mean survival time* (months)	Incidence of leukaemia at 18 months		
			Control	After 1,500 r.	After 1,000 r.
CBA	M	25	Nil	8/8	3/10
CBA	F	27	Nil	10/12†	14/20
F_1(CBA × A)	F	29	Nil	7/20‡	3/10
F_1(A × CBA)	F	18§	Nil	8/10	3/10
A	F	19§	1%	6/10	4/15

* Measured from 8–10 weeks of age when the course of radiation started.

† Acute radiation deaths (8) occurred in this group only. These deaths occurred near the end of the course of irradiation either just before or during the few days after the last dose, and were apparently due to severe anaemia resulting directly from bone-marrow damage caused by the irradiation.

‡ This group received 25 r. daily, not 50 r.

§ The shortened survival time is due to the high incidence of milk-factor mammary tumours.

the dorsum between the scapulae and in the neck, without involvement of the iliac lymph nodes and with a very variable degree of visceral involvement. In myeloid leukaemia the lymph nodes are not conspicuously enlarged and may show a green or brown-green tinge: the spleen also is grey-brown rather than creamy. The accurate classification of stem-cell leukaemias where the abnormal cells are not accompanied by more mature and definitely myelocytic or lymphoid cells requires methods additional to the histological examination of tissue. There is a very distinctive generalized reticulo-sarcoma of liver and spleen accompanied in middle-aged, but not aged, mice by involvement of the vagina: this seems to be the same as the histiocytoma described by Gorer (1946). The localized proliferative diseases of the mesenteric lymph node are uncommon; this is helpful since they can be difficult to classify histologically and their clinical importance is uncertain. Neither the mesenteric lymph-node diseases nor the histiocytoma of liver and spleen have been included in the class of murine leukaemia. Some cases where the cell type is that of the histiocytoma, but the lymph nodes and/or bone-marrow are involved, have been considered to be leukaemic.

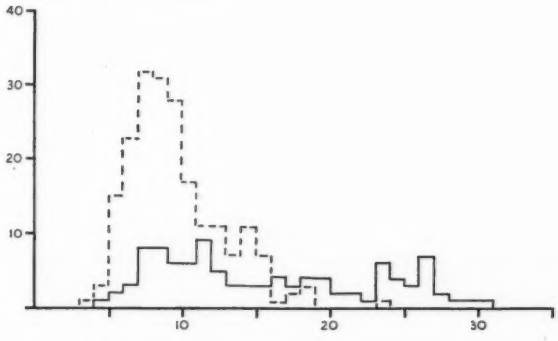
2. Radiation-Induced Leukaemia

In order to assess the leukaemogenic effect of irradiation, mice aged 8–10 weeks were given *x* rays either in single doses or in a series of equal doses five days a week, once weekly or once fortnightly, to predetermined total doses (Corp, 1957; Mole, 1955, 1956). Table I shows that a 6-week period of daily irradiation at 50 r. daily 5 days a week can produce a leukaemia incidence of 60–100% in three genetically different mouse strains, none of which has a high spontaneous incidence. This type of exposure to radiation seems therefore to be uniformly leukaemogenic in the mouse, and the results suggest that every mouse is capable of developing leukaemia if the right conditions for irradiation are chosen.

No comparable human experience exists, although the *x*-ray treatment of ankylosing spondylitics is not too dissimilar in length of over-all exposure time and in accumulated dose. The major difference is in the irradiation of the whole of the mouse body as compared with the more localized spinal irradiation of the human patients. Kaplan & Brown (1951) showed experimentally, by irradiating the two halves of the mouse's body at differing times, how necessary simultaneous or nearly simultaneous irradiation of the whole body was if a very high incidence of thymic leukaemia was to be produced. Whole body irradiation of mice with 1,000–1,500 r. over 4 to 6 weeks (Table I) results in an incidence of leukaemia a hundred times greater than in subjects of ankylosing spondylitis given the same dose to the spinal marrow.

Any estimate of the degree of leukaemogenesis by radiation depends on the base-line of incidence among controls. Before 18 months this was negligible (Table I) but, as the control mice aged beyond the 18-month point, proliferative diseases of reticular tissue began to occur. Their total frequency, however, did not exceed 15–20% in any of the strains of Table I, so that irradiation clearly did more than merely shorten the " maturation " time of a spontaneously occurring disease. Nevertheless the incidence of radiation-induced leukaemia against time after irradiation is clearly an important question, and in fig. 1 this is shown for over 300 examples occurring in irradiated female CBA mice. The leukaemias are divided into two classes, thymic leukaemia and generalized leukaemia of all other types. The class of thymic leukaemias includes a majority in which the thymus, or the superior mediastinum when thymus and paratracheal lymph nodes were not separately identifiable, was involved to no greater an extent than might have been expected in a proliferative disease affecting the whole lymphoid system. The distinction between these and the generalized lymphoid leukaemias depended on whether the thymus was or was not involved as judged by the unaided eye, and was perhaps arbitrary. In old mice the thymus is naturally so atrophic that its involvement in proliferative disease might not be expected. In some young adult mice, which had been irradiated and had died from leukaemia, the thymus had apparently not yet properly regenerated after the damage produced by the irradiation. The paratracheal lymph nodes were then always involved, and often the leukaemic cells were infiltrating the paravertebral

FIG. 1. DISTRIBUTION OF LEUKAEMIA IN FEMALE CBA MICE PLOTTED AGAINST TIME AFTER START OF IRRADIATION



Abscissae: months after start of irradiation
Ordinates: number of cases

Mice were aged 8–10 weeks at the beginning of their exposure.
Broken line: thymic leukaemia
Continuous line: other leukaemias

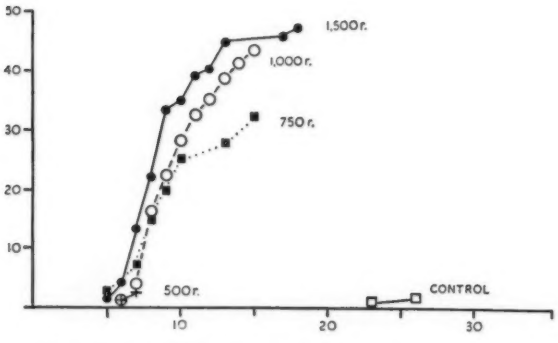
muscles of the thorax and the spaces around the nerve-roots and the spinal cord itself, thus causing gross paralysis of the lower trunk and hind limbs.

Figure 1 shows that an observational period of 19 months revealed all but one of 204 thymic leukaemias produced by irradiation of young adult female CBA mice. Generalized leukaemias, however, went on occurring for 30 months at least and did not show such a sharp peak of incidence during the first year. Furth & Upton (1954) found a similar differential incidence with age in Rfl mice given a single dose of *x* rays. Leukaemia of either kind did not appear before 21 months in controls (figs. 2, 3).

The cumulative incidences with time of the two classes of leukaemia are given separately in figs. 2 and 3 where the effects of different total doses are also shown separately. In these experiments the dose was 10 r. daily or 50 r. weekly, 25 r. daily or 125 r. weekly, or 50 r. daily (over-all exposure time 2–30 weeks). Larger individual doses reduce the over-all incidence of leukaemia (Table II and Mole, 1956, 1958). Figure 2 shows a striking threshold effect. Very little thymic leukaemia was produced by a total dose of 500 r. The larger total of 750 r. increased the incidence more than tenfold, but increasing the dose still further did not increase the yield commensurately.

Figure 3 shows a very different picture. The incidence of generalized leukaemia (all non-thymic types combined) steadily increased with time whatever the total dose. Late in life the lowest total dose, 500 r., apparently had more effect than the higher doses, but after 20 months there were very few mice left in the other groups. It must be emphasized that this group of generalized leukaemias is not homogeneous, and needs further analysis: for example, after total doses of 1,500 r., a high proportion of cases of aplastic anaemia, which after review were considered to be cases of leukaemia, occurred just as in the patients with ankylosing spondylitis (Court-Brown & Doll, 1957).

FIG. 2. DOSE OF *x* RADIATION AND CUMULATIVE INCIDENCE OF THYMIC LEUKAEMIA IN FEMALE CBA MICE



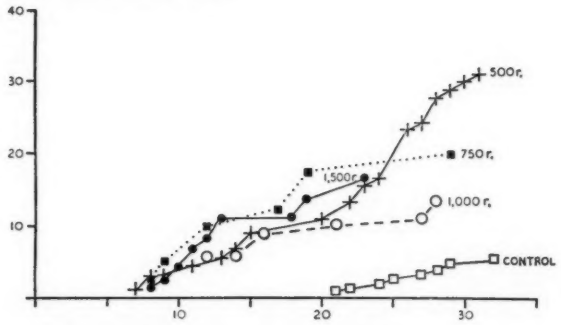
Abscissae: months after start of irradiation
Ordinates: percentage of mice with thymic leukaemia

The number of mice in a group was 72, 115, 40 and 90, for the total doses of 1,500, 1,000, 750 and 500 r. respectively.

Over-all exposure time: 2–30 weeks (see text). Each accumulated total dose is indicated by a different symbol:

- ●–● total dose of 1,500 r.
- ○–○ total dose of 1,000 r.
- ■–■ total dose of 750 r.
- +–+ total dose of 500 r.
- □–□ control animals

FIG. 3. DOSE OF *x* RADIATION AND CUMULATIVE INCIDENCE OF NON-THYMIC LEUKAEMIA IN FEMALE CBA MICE



Abscissae: months after start of irradiation
Ordinates: percentage of mice with non-thymic leukaemia

Data as for fig. 2.

The excess of leukaemias produced by 500 r. (fig. 3) may perhaps be exaggerated by excluding from the class of leukaemia the reticulo-sarcomata of liver and spleen mentioned earlier. The incidence of this type of lesion was markedly reduced after all the radiation doses considered here. If one effect of radiation is to encourage the generalization of an otherwise focal proliferation of reticular tissue, it may not be fair, when assessing leukaemogenesis, to include the generalized cases (which may occur in the irradiated) but to exclude the focal cases (which occur in the controls). Clearly much more analysis is needed to see whether the best basis for classification is clinical picture, morbid anatomy or cytological character, and before deciding whether the leukaemic diseases are a unitary group or have diverse aetiologies.

3. The Role of the Thymus

Comparison of figs. 2 and 3 shows how easy it may be to reach erroneus conclusions about the dose-response curve for the induction of leukaemia by radiation, when attention is confined to thymic leukaemia or when the observational period is cut short. Unfortunately most experimental work on murine leukaemia has been confined to relatively short-term experiments on thymic leukaemia. Here the evidence that radiation causes thymic leukaemia by an indirect mechanism

TABLE II. INCIDENCE OF LEUKAEMIA IN IRRADIATED FEMALE CBA MICE AND SIZE OF INDIVIDUAL DOSE

Total dose of *x* rays	Number of mice with leukaemia/number in group					
	25 r. daily	125 r. weekly	250 r. fort-nightly	50 r. daily	250 r. weekly	500 r. fort-nightly
1,000 r.	10/18	12/20	3/18	14/20	9/19	4/15
1,500 r.	11/20	18/20	5/17	—	—	—

is strong (Kaplan, 1954; Kaplan, Carnes, Brown & Hirsch, 1956), and the remarkable effect on the incidence of thymic leukaemia of increasing the total dose from 500 to 750 r. (fig. 2) would be consistent with this view. Either thymic leukaemia is something different from other leukaemias or the thymus is conspicuously involved in murine leukaemia only when the physiology of the mouse is sufficiently disturbed, as by a large enough dose of radiation given over a period of time.

The central place of the thymus in recent studies on murine leukaemia is clearly shown in recent reviews (Kaplan, 1954; Upton & Furth, 1957). However, it may not be right to attribute a peculiar importance to the thymus. It represents a large fraction of the lymphoid tissue of the young mouse so that thymectomy, regarded simply as removal of lymphoid tissue, might be expected to reduce the incidence of diseases arising in such tissue, and the restoration of susceptibility to leukaemia, spontaneous or radiation-induced, by the subcutaneous grafting of thymuses into thymectomized animals has not in fact been controlled in published experiments by the grafting of equal masses of non-thymic lymphoid tissue. This is unfortunate because the demonstration that leukaemia can apparently originate in an unirradiated thymus, when this has been implanted into a thymectomized mouse after it has been irradiated (Kaplan *et al.* 1956), would seem to prove a humoral mechanism for leukaemogenesis. The proof hinges on the genetic characters of the leukaemia as determined by transplantation. Further evidence is being sought in this laboratory by a different method, chromosome markers being used to identify the origin of the leukaemia (Ford, Ilbery and Loutit, unpublished observations from this laboratory).

4. Dose-Rate and the Leukaemogenic Mechanism

Further information on the over-all incidence of leukaemia after total doses of less than 500 r. is clearly needed before the shape of the dose-response curve may be guessed at, and it is possible that other observations may allow a decision about the hypothesis that leukaemia is produced by radiation through the production of point mutations. The chromosome complement of leukaemic cells in the mouse is so commonly abnormal in several respects (Ford, Hamerton & Mole, 1956),

TABLE III. THE EFFECT OF DOSE-RATE ON THE INCIDENCE OF LEUKAEMIA IN FEMALE MICE GIVEN 1,000 r. WHOLE BODY γ IRRADIATION DURING A 4-WEEK PERIOD

Group	Irradiation schedule	Dose-rate (r./hour)	Number of mice with leukaemia/number in group after 10 months' observation	
			CBA	C57 black
A	5 days weekly	81	4/10	4/9
B	5 days weekly	21	0/10	4/9
C	5 nights weekly	3.3	0/10	0/9
D	Continuously	1.3	0/10	0/5
E	Controls	Nil	0/10	0/8

The dose in Group D was 900 r., not 1,000 r. as had been planned. (Similar results are being obtained in other groups of mice similarly irradiated but observed so far for only 5–6 months.)

that it is tempting to think that these visible abnormalities indicate that the basic change which makes cells leukaemic is not a point mutation but something grosser. If such gross changes are indeed an aetiological factor in leukaemogenesis the effect of dose-rate might be of overriding importance. This deduction is being confirmed by the preliminary results of an experiment designed to test the question, in which the same total dose of radiation spread out over the same over-all exposure time was given in daily doses at different dose-rates (Table III). Because of the short observation period any conclusions can at present apply only to thymic leukaemia, but for this form of leukaemia it is clear that somatic mutation, if it occurs, is of subsidiary importance to some factor dependent on dose-rate. Much more work is needed to extend these observations to smaller total doses spread out over longer periods of time and to other kinds of leukaemia. Nevertheless, chromosomal abnormalities in leukaemia cells were, if anything, commoner in generalized leukaemia than in purely mediastinal leukaemia.

This account is a personal one: few of the many published papers have been selected for individual reference. Excellent reviews of the vast field of murine leukaemogenesis have been made by others (Furth, 1946, 1951; Kaplan, 1954; Upton & Furth, 1957).

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CARCINOGENESIS OF SKIN TUMOURS INDUCED BY RADIATION

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1 Induction time and tumour yield
2 Fractionation of dosage
3 Field size
4 Dose
5 Tumour types
6 Histogenesis
References

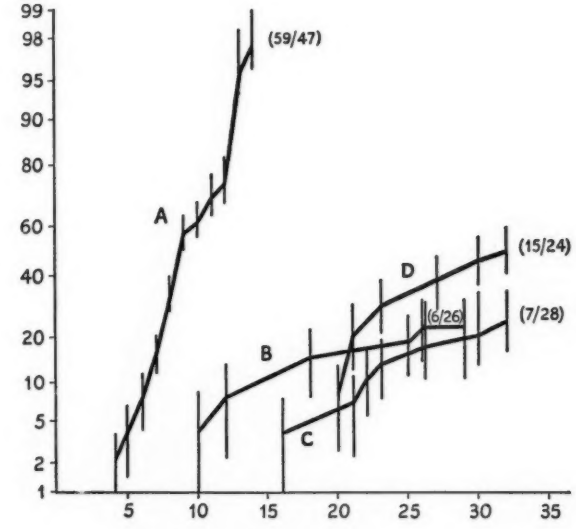
The carcinogenic action of ionizing radiation was recognized soon after the first medical application of *x* rays and confirmed experimentally in 1910 (Marie, Clunet & Raulet-Lapointe, 1910). The realization of the cancer hazard has led to the formulation of protective measures, and the incidence of untoward sequelae in the skin has substantially decreased. Little progress was made in the experimental study of the carcinogenic action of penetrating radiations on the skin, until the use of electron beams and of β rays from radioactive sources made it possible to limit the action of irradiation in small laboratory animals to the skin without causing injuries to deep-seated organs.

The use of radiations for the experimental induction of tumours has some advantages over that of the chemical carcinogens, as measured doses can be given to circumscribed regions. This makes it possible to obtain more precise quantitative data on dose-response curves, on fractionation of dosage and on field size than is usually feasible with the application of chemical carcinogens. The main disadvantages of radiations as carcinogenic agents are the long induction period, the incidental loss of experimental animals from unrelated causes, and the low yield of tumours compared with those obtained with the chemical carcinogens, which make the interpretation of precancerous changes in the skin difficult.

1. Induction Time and Tumour Yield

A comparison of induction time and tumour yield is given (curve A) for rats treated with a chemical carcinogen (a 1% solution of 9 : 10-dimethyl-1 : 2-benzanthracene painted once weekly on the skin) and (curve B) for rats exposed to an electron beam (generated at 1.0 MeV; a single dose of 12,000 rads was delivered to a circular skin region of 2.5 cm. diameter in 20 seconds) (see fig. 1). To obtain an incidence of 20% of tumours in rats requires about 19 months longer for the irradiated than for the chemically treated groups. The tumour yield in the chemically treated group is 98 ± 2% after 13 months as against 23 ± 8.5% after 26 months in the irradiated rats. If the number of tumours per animal is considered, the difference becomes even greater: there is an average of 1.3 tumours per rat at risk after chemical treatment and of 0.23 per rat after irradiation. Even so, the estimate for the chemical

FIG. 1. RATES OF TUMOUR INDUCTION IN RATS, GIVEN IN CUMULATIVE PERCENTAGES WITH STANDARD ERROR



Abscissae: time (months)
Ordinates: rats with tumours (%)

Curve A: following weekly applications of a 1% solution of 9:10-dimethyl-1:2-benzanthracene in acetone

Curve B: following a single exposure of a circular field of 2.5 cm. diameter, to an electron beam generated at 1.0 MeV. A dose of 12,000 rads was delivered in 20 sec.

Curve C: following two equal exposures at an interval of two months, of a circular field of 2.5 cm. diameter, to an electron beam generated at 0.7 MeV. Each dose of 2,300 rads was given in 3.3 sec.

Curve D: following a single exposure of a circular field of 2.5 cm. diameter, to an electron beam generated at 0.7 MeV. The dose of 12,000 rads was delivered in 20 sec. Simultaneously an anterior and a posterior field of about 10 cm.² were exposed to a dose decreasing from 2,000 rads to nil.

The figures in brackets at each curve (e.g., 59/47) give the number of tumours in the total number of animals at risk.

group may err on the low side, since only histologically different tumours (sarcomata and carcinomata) or malignant tumours of the same histological type well separated by normal tissue are counted as multiple tumours.

2. Fractionation of Dosage

The greater effectiveness of the chemical carcinogen shown in fig. 1 as compared with irradiation might be attributed to the fact that irradiation was given in a single dose while the carcinogen was applied once weekly for a number of months. Henshaw, Snider & Riley (1949) have exposed rats to whole surface irradiation from β rays daily for months, and another group to single doses. After 19 months of daily exposures to 50 rep (a total dose of about 24,600 rep), the tumour incidence was about equal to that found 15 months after a single dose of 4,500 rep, while single doses of 6,500 rep induced even more tumours. Exposures at daily doses of 0.5 and 5.0 rep failed

to increase the incidence of skin tumours. In these experiments, therefore, single exposures were more efficient as carcinogenic agents than repeated daily exposures. Depending on dose-level and interval, fractionation of dosage may also prove more effective than single exposure. In the figure, curve C shows the results in a group of rats exposed to two doses of 2,300 rads given at an interval of two months. The tumour yield for a total dose of 4,600 rads equals that shown in curve B for a single dose of 12,000 rads.

3. Field Size

A single dose of 2,300 rads given over a field of 2.5 cm. diameter failed to induce any tumours in rats in our experiments. Single doses of 660–1,000 r. of *x* rays given to rats as whole body exposures produced skin cancers in 5–16% of the surviving animals (Koletsky & Gustafson, 1955; Brecher, Cronkite & Peers, 1953; Finerty, Binhammer, Schneider & Cunningham, 1953). In addition multiple fibromata of the dermis and subcutaneous tissue were noticed in about 33% of such rats (Koletsky & Gustafson, 1955). The carcinogenic dose in these experiments was well below the dose necessary to induce tumours in small irradiated regions.

In whole surface irradiation with β rays (Henshaw *et al.* 1949), the yield of multiple tumours was 2.1 per rat at 11 months and 6.6 per rat at 15 months after a single exposure to 4,500 rep. This contrasts with an average tumour yield of 0.25 per rat as shown in curve C of fig. 1, which illustrates the results following a dose of 4,600 rads given over a circular field of 2.5 cm. diameter. If the field size is increased the tumour yield rises. Curve D in fig. 1 shows the results in a group of rats which received, in addition to a dose of 12,000 rads over a central circular area of 2.5 cm. diameter, doses decreasing from 2,000 rads to nil over separate anterior and posterior fields of about 10 cm^2. The percentage of tumour-bearing rats was 50 ± 10.2 and the average yield of tumours per rat at risk 0.62. Of these tumours 40% arose in the region which was irradiated with at most 2,000 rads, but in another experiment a dose of 2,300 rads over a 2.5 cm. field failed to induce tumours.

Within a given field a uniform distribution of radiation energy appears to be more effective in producing carcinogenesis than concentration of the same energy into a number of smaller foci (Passonneau & Hamilton, 1951).

4. Dose

If mutation of individual cells induced by irradiation was the primary mechanism in the induction of tumours by irradiation, a dependence of tumour yield on dosage but not on fractionation of dosage might be expected from analogy with mutation in germ cells, but dependence on fractionation has been shown above. Even for the same dose-rate, the relationship between dose of irradiation and tumour yield is by no means simple.

In the experiments of Henshaw *et al.* (1949) the yield of cutaneous tumours at 11 months was 3.1, 3.0 and 2.0 per rat for doses of 4,500, 6,500 and 8,500 rep respectively, and at 15 months 6.7, 7.6 and 3.7 tumours per rat for the same dose-levels. The claim by Schubert, Künkel, Overbeck & Uhlmann (1956) of a logarithmic relationship between dose of irradiation and yield of tumours is not supported by their evidence. The number of animals used in their experiments is too small for a significant difference to be deduced between tumour yields of 70%, 75% and 80% respectively at three dose-levels.

5. Tumour Types

Chemical carcinogens applied to the skin of mice and rats induce predominantly carcinomata and less frequently sarcomata. In experiment A of fig. 1, 73% of the tumours were carcinomata and the remaining 27% sarcomata. For experiments B, C and D the proportion of carcinomata dropped to 14% and that of sarcomata rose to 86%. Very similar figures were collected from the literature for mice (Glücksmann, Lamerton & Mayneord, 1957). The method of application of both irradiation and chemical carcinogens and also the site of treatment influence the histological type of the tumours. Schubert *et al.* (1956) found carcinomata in 61% of tumours following subcutaneous insertion of radioactive pearls and 84% following intraperitoneal insertion, while Glücksmann & Cherry (1958) obtained 4% carcinomata and 96% sarcomata in the vagina after local application of 9 : 10-dimethyl-1 : 2-benzanthracene, whereas in the same animals all the tumours at the vulva were carcinomata. The presence of hair follicles and the absence of a cellular scar seem to favour the formation of carcinomata, and the reverse seems to hold true for the appearance of sarcomata. The depth of the initial radiation burn in relation to the thickness of skin structures also influences the histological result and accounts for the appearance of carcinomata rather than sarcomata in human skin as opposed to that of rats. In the human skin, too, malignancies appear at the edges of ulceration and are related to impaired repair processes (Teloh, Mason & Wheelock, 1950).

6. Histogenesis

Radiation has a primarily destructive effect on the skin in the doses given to induce cancers. The extent and degree of injury vary with the dose, the size of the field and the depth of penetration of the radiation. The radiation burn involves epidermis, hair follicles and dermis, and with higher voltage extends down to the region of the panniculus carnosus in the thin skin of the mouse and stops short at the supra-pannicular tissue in the rat. The radiation burn takes about ten days to develop and the necrotic tissue is then sloughed and scab formation follows. Healing is due to an inflammatory reparatory reaction which in the mouse takes place in the subcutaneous tissue and bulges upward and in the rat involves the supra-pannicular tissue and lower dermal regions. The dermal tissue even at the sides of the burn contributes little to scar formation. Epithelial regeneration is due to migration of neighbouring epidermal cells. By the end of the first month the burn may appear healed but consists histologically of a poorly developed scar tissue with round-cell infiltration, oedema and endarteritic and periphlebitic changes. The vascular lesions are progressive and cause hyalinization of the scar tissue which in the mouse becomes almost acellular and avascular and is again shed after detachment of the epidermis by oedematous changes. The new ulcer is repaired in the same unsatisfactory fashion, and the process is repeated in the mouse until, after about 14 months, the epidermis at the edge of the ulcer undergoes malignant change.

In the rat, as in the mouse, the scar is unstable and appears as an atrophic lesion or in other phases as hyalinized keloidal tissue situated close to the panniculus carnosus. At the periphery of the scar or in the early reparatory phase following ulceration, marked fibroblastic activity is noticed after 18 months and later, and a somewhat more cellular scar is formed which in turn undergoes malignant change to form a

sarcoma. In both the rat and mouse, carcinomata develop at the edge of ulcers.

Radiation thus causes destruction of the irradiated tissue, permanent epilation and vascular changes which interfere with the production of a stable scar. Tumours form in the regenerating tissue which replaces that destroyed by direct irradiation, and they usually appear in one of the later of the repeated regenerative cycles. In the case of the sarcomata in the rat it is quite likely that the malignant change occurs in cells whose ancestors were not directly exposed to irradiation but which have subsequently migrated into the irradiated region.

By contrast chemical carcinogens stimulate growth in the epidermis and hair follicles, lead to the production of hyperplasia and warts and are followed by an early malignant change in the offspring of directly treated cells. Sarcoma formation is due to stimulation of the dermal tissue by the carcinogen which may come into contact with it through a secondary ulceration following the appearance of warts or carcinomata. These differences in the mechanism of action explain the longer induction period in radiation-induced than in chemically-induced skin cancers and also the scarcity of carcinomata as compared with sarcomata. After chemical treatment carcinomata arise not only from the epidermis but also largely from the hair follicles (Wolbach, 1951) which are destroyed by irradiation. On the other hand the demands of unstable scars on the reparative functions of connective tissue call for increased cellular activity and facilitate the malignant change in the presence of the unfavourable conditions created by the irradiation. Treatment with chemical carcinogens of a scar induced by irradiation induces predominantly sarcomata and only later a few carcinomata (Boag & Glücksmann, 1956) which may have originated outside the irradiated region and invaded it subsequently.

Regenerating tissue seems particularly sensitive to malignant change after irradiation. Shielding of the thymus in whole body irradiation suppresses the incidence of lymphomata originating in the thymus. Removal of the thymus during whole body exposure and re-transplantation of the unirradiated thymus cause tumours to appear in the implanted gland (Kaplan & Brown, 1954); this is attributed to the reaction of regenerating cells in the grafted thymus to the systemic changes induced by irradiation.

The abnormal sensitivity of regenerating cells to radiation may also account for the induction by small doses of radiation of sarcomata in skin lesions caused by the injection of silicates or of *Streptobacillus caviae* (Lacassagne & Vincent, 1929; Burrows, Mayneord & Roberts, 1937).

Stimulation of regenerating epidermal cells and hair follicles by the painting with croton oil of irradiated mouse skin may account for the appearance of some warts following small doses of irradiation (Shubik, Goldfarb, Ritchie & Lisco, 1953). These warts did not progress to malignancy and the tumour yield was smaller than after similar painting experiments in mice following thermal burns (Saffiotti & Shubik, 1956). In experiments on mice treated with croton oil after large doses of irradiation by an electron beam, we obtained some warts after about two months but they all regressed and were not followed by malignant change. In rats even repeated thermal burns failed to induce any skin tumours in our experiments.

This short review may serve to emphasize that carcinogenesis of the skin induced by irradiation is a rather complex process of long duration and relatively low efficiency. The development of skin cancers appears to be related to the severity of the burn and its influence on the subsequent regenerative processes. There seems to be an optimal degree of radiation injury for carcinogenesis intermediate between lesions insufficient to upset repair and too severe for any effective attempt at repair (Glücksmann *et al.* 1957). Thus irradiation seems to create environmental conditions for malignant change in regenerating cells rather than directly to induce a malignant change in the irradiated cells and their offspring.

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EXPERIMENTAL INDUCTION OF TUMOURS OF THE THYROID BY RADIATION

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1 The TSH factor in thyroid tumour induction
2 Irradiation dosage to the thyroid from ^{131}I
3 Radiation-induced thyroid tumours in rats
4 Morphology of experimentally induced thyroid tumours
5 Application of experimental findings to man
References

During the past ten years many thousands of patients with hyperthyroidism have been treated with radioactive iodine (^{131}I), with an average irradiation to the thyroid of 10,000–15,000 rads.[1] Although this dosage is in the carcinogenic range for the skin of experimental animals, no thyroid carcinomata have so far been reported in these patients. But one cannot assume that the thyroid is resistant to the carcinogenic action of radiation, since an association has now been established between the development of thyroid cancer in late childhood or early adult life and previous moderate irradiation of the thyroid in infancy or early childhood (given for treatment of an enlarged thymus or cervical lymph nodes or tonsils) (Duffy & Fitzgerald, 1950; Simpson, Hempelmann & Fuller, 1955; Clark, 1955; Kilpatrick, Blomfield, Neal & Wilson, 1957; Simpson & Hempelmann, 1957). As little as 200 rads *x* rays to the thyroid has proved carcinogenic in these circumstances. The problem of the risk of developing thyroid cancer after ^{131}I therapy and the paradoxical carcinogenic effects of high and low dosage of irradiation have been a stimulus to experimental studies on rats, which are described below.

1. The TSH Factor in Thyroid Tumour Induction

Tumours of the rat's thyroid have been induced by a variety of techniques, reviewed by Bielschowsky (1955) with full bibliography: diets deficient in iodine, goitrogen administration, administration of the carcinogen 2-acetamidofluorene (AAF) together with or before goitrogen treatment, AAF and partial thyroidectomy. Griesbach, Kennedy & Purves (1945), having shown that goitrogens lead to increased secretion of pituitary thyroid-stimulating hormone (TSH), concluded that tumour induction results from maintained TSH stimulation and not from any direct carcinogenic action of goitrogens on thyroid cells. All these procedures cause prolonged stimulation of the thyroid by TSH. The sequence of events in these experiments has been rapid goitrogenesis, adenoma formation after 8 months and carcinomata after 20 months. The combination of the carcinogen AAF given together or as pre-treatment with prolonged goitrogen markedly speeded up the development of adenomata and carcinomata. Hall (1948) postulated that AAF "initiated" neoplastic cells which were "promoted" to tumour formation by the goitrogen-induced TSH stimulation.[2] This attractive hypothesis is not immediately applicable to tumour formation by goitrogens or iodine deficiency acting on their own. A two-stage mechanism under these circumstances implies the existence of an unknown "initiating" carcinogen present in all laboratory environments or diets, or that all rat thyroids contain inherited "initiated" cells, or that TSH is an "initiating" as well as "promoting" carcinogen. Since the thyroid cells of the adult rat or human being normally divide very rarely (Leblond & Walker, 1956), one must also consider the possibility that the enforced series of mitotic divisions of these cells that results from goitrogen treatment may of itself lead to neoplasia by a mechanism yet to be elucidated. Nevertheless, it must be emphasized that pre-treatment with AAF leads to a striking intensity of adenoma formation at a time when control animals on goitrogen alone show only simple hyperplasia of their thyroids (Bielschowsky, 1945).

2. Irradiation Dosage to the Thyroid from ^{131}I

In most of the experiments to be citted, ^{131}I was given as sodium iodide in water, carrier free, injected intraperitoneally in doses varying from 5 to 400 microcuries (μc) per animal. To find the mean dose in μc per unit mass of thyroid tissue, it is necessary to know the thyroid weight, the thyroid uptake of ^{131}I, and its rate of uptake and loss from the gland, and to allow for the radioactive decay of the isotope. The concentration of ^{131}I in the rat's thyroid reaches a maximum in 16–36 hours and falls exponentially from about 36–48 hours. The biological half-life of the ^{131}I in the thyroid is determined by daily in-vivo counts of thyroid radioactivity for 7–10 days (Maloof, Dobyns & Vickery, 1952). In the calculation of the mean thyroid dose of irradiation in rep[3] (röntgen equivalents physical) or in rads, in the small thyroid of the rat, only the β rays from ^{131}I are considered. The reader is referred to Feller, Chaikoff, Taurog & Jones (1949) and to Maloof *et al.* (1952) for formulae and discussion of the calculation. These measurements are essential because the avidity of the thyroid for iodine varies considerably, and this avidity is inversely proportional to the iodine in the diet and the environmental temperature. Thus the maximum uptake of ^{131}I into the thyroid has varied from 15% to 60% in different laboratory regimes. Although one can arrive at an approximation of the mean radiation dose to the thyroid, the range of radiation within the gland is wide and not precisely known. Much of the β-ray energy which originates in the peripheral follicles and isthmus escapes from the gland. The ^{131}I uptake of the peripheral follicles of the rat's thyroid is less than that of the central ones (Leblond & Gross, 1948) and there is a further variation in ^{131}I uptake between follicles within the same lobule. Similar variations in uptake occur in man. It is likely that the dose of ^{131}I which must be given to suppress cellular activity throughout most of the

[1] See also Wayne, Macgregor & Blomfield, *Brit. med. Bull.* 1952, **8**, 148.—Ed.

[2] See also Salaman, p. 116 of this number of the Bulletin.—Ed.

[3] "An unofficial unit, proposed by H. M. Parker, of amount of radiation of any kind which yields an amount of energy transferred to the tissue equal to that transferred by 1 roentgen of hard x- or γ-radiation (200 Kv. or greater)." *Dorland's illustrated medical dictionary*, 23rd ed., 1957.—Ed.

gland, i.e., reach the lowest effective level of the range, must inevitably submit parts of the thyroid to an unnecessarily high and possibly carcinogenic level of irradiation.

3. Radiation-Induced Thyroid Tumours in Rats

The development of benign and malignant tumours in the rat's thyroid 1½–2 years after a single injection of 400 μc ^{131}I was reported by Goldberg & Chaikoff (1952). The mean thyroid irradiation of these rats should have been more than 150,000 rads according to the calculations of Feller *et al.* (1949). Later experiments (Doniach, 1953; Lindsay, Potter & Chaikoff, 1957; Field, Valentine, Bernick, Orr & Starr, 1956) showed, however, that this order of irradiation inhibits thyroid tumour formation; the optimal carcinogenic dose range of ^{131}I is much lower. Lindsay *et al.* (1957) treated a large series of rats at the age of 6–12 weeks with single injections of varied doses of ^{131}I. Of 20 rats given 25 μc and killed at 28 months of age, thyroid adenomata were found in nine and follicular or papillary carcinomata in three. A carcinoma was also present in 1 of 6 rats given 10 μc and killed at 32 months and another carcinoma in 1 of 10 rats given 100 μc and killed at 31 months. Adenomata were present in some of the thyroids in both these groups. No follicular or papillary carcinomata were found in 146 rats given 400 μc and killed at 2–2½ years, nor in 16 rats given 200 μc and killed at 32 months, nor in 156 untreated controls killed at 24 to 32 months. My findings (Doniach, 1950, 1953, 1956) are summarized in Table I. An injection of 30 μc ^{131}I, calculated to give a mean dose of 10,000–15,000 rads to the thyroid (comparable with the treatment of hyperthyroidism), was given at the age of 2–3 months, and the rats were killed 13–15 months later. The experimental conditions lasted just over a third rather than two-thirds of the rats' lifetime, a duration well below the time threshold required for the production of carcinomata by goitrogens. The ^{131}I produced adenomata in 40% of the rats, but no carcinomata. Prolonged post-irradiation treatment with methylthiouracil, given in the drinking-water, induced multiple adenomata in over 90% of the animals. The tumours almost replaced one or both thyroid lobes in over 50% of the rats. Carcinomata were found in 11 of the 48 animals so treated. Adenomata were present in 80% of unirradiated rats killed after 15 months of methylthiouracil treatment; none of their thyroids showed adenomatous replacement or carcinomata.

In order to gauge whether the lower end of the thyroid radiation range—2,000–20,000 rads—in the above experiments was carcinogenic, a series of rats were given 1,100 rads external irradiation to the thyroid with 190 kv *x* rays. In contrast to irradiation from ^{131}I, irradiation from *x* rays is uniformly distributed in the thyroid. The data (Table I) show almost the same quantitative carcinogenic effects of 30 μc ^{131}I and 1,100 rads *x* rays. Although ^{131}I irradiation consists of β rays given over a few days, and external irradiation consists of *x* rays given in a few minutes, it is unlikely that their different quality and duration would account for a tenfold difference in dose response. It is more likely that the lowest part of the dosage range in the thyroid, possibly 2,000–4,000 rads, is effective in adenoma production and in summation with goitrogen to produce carcinomata. The combination of ^{131}I followed by methylthiouracil paralleled the action of AAF followed by goitrogen. Thus the initial radiation may be regarded, like AAF, as having "initiated" neoplastic cells which were "promoted" to tumour formation by the goitrogen-induced TSH stimulation.

TABLE I. INCIDENCE OF THYROID TUMOURS IN RATS KILLED 15 MONTHS AFTER IRRADIATION OF THE THYROID, SOME ANIMALS BEING MAINTAINED THEREAFTER ON METHYLTHIOURACIL IN THE DRINKING-WATER

Treatment	Total number of rats	Number of rats with thyroid adenomata 0.5 mm. or more in diameter	Number of rats with adenomatous replacement of one or both thyroid lobes	Number of rats with thyroid carcinoma
Nil . . .	41	0	0	0
Methylthiouracil alone . .	50	39	0	0
30 μc ^{131}I (15,000 rads to the thyroid) . .	52	21	0	0
30 μc ^{131}I plus methylthiouracil	48	47	27	11
1,100 rads *x* rays to the thyroid .	13	4	1	1
1,100 rads *x* rays to the thyroid plus methylthiouracil	22	21	17	7

4. Morphology of Experimentally Induced Thyroid Tumours

The radiation-induced tumours showed a microscopic appearance identical with those produced by goitrogen and iodine deficiency, described and illustrated in numerous publications. They are similar to human tumours and show the same variants: macrofollicular, microfollicular, trabecular, papillary and undifferentiated. Carcinomata are distinguished by infiltration of surrounding tissues, or by permeation of extra-thyroidal veins, or by the presence of pulmonary metastases. The malignant thyroids were mostly larger and heavier than the non-cancerous ones in the same group. Of 18 thyroid cancers in 70 rats (Doniach, 1950, 1953, 1956), 13 occurred in 30 females and 5 in 40 males. ^{131}I proved a more potent initiating agent than AAF, in the doses used, judged by comparison of the number of adenomata promoted by subsequent goitrogen treatment (Doniach, 1950).

Lindsay *et al.* (1957) have recently pointed out that tumours, readily differentiated from neoplasms induced by radiation or goitrogen, may be found in the thyroids of old untreated rats. They considered them to be non-invasive, non-metastasizing low-grade alveolar carcinomata. These were called "microadenomas" by Doniach (1953), and γ nodules by Axelrad & Leblond (1955), who regard them as benign neoplasms relatively independent of TSH.

5. Application of Experimental Findings to Man

There is indirect evidence from changes observed in pituitary cytology (Goldberg & Chaikoff, 1950; Doniach, 1953) and from measurements of mean thyroid cell height (Doniach & Logothetopoulos, 1955) that ^{131}I irradiation of the thyroid causes a prolonged rise in TSH secretion. This occurs not only after necrotizing doses of ^{131}I, but also to a minor degree after 30 μc ^{131}I. Thus ^{131}I may be considered not only to initiate neoplastic thyroid cells, but in addition to cause promotion of tumours by its effect on TSH secretion. The TSH secretion is proportional to the degree of thyroid damage which is in turn proportional to the dose of ^{131}I. However, heavy doses of ^{131}I inhibit the subsequent thyroid growth response to TSH (Skanse, 1948; Maloof *et al.* 1952; Doniach, 1953; Potter, Taurog & Chaikoff, 1956) and thus neutralize the promoting effect of the raised TSH. To induce thyroid tumours with ^{131}I alone, a dose must be given which initiates neoplastic cells and strikes an optimal balance between rise in TSH and depression of thyroid cell growth response. The rise of TSH following exposure of the thyroid to 15,000 rads from ^{131}I is minimal compared with that induced by goitrogens. This finding in rats is likely to obtain in hyperthyroid patients treated with ^{131}I. Although neoplastic cells are probably initiated in the thyroids by the therapy, this radiation is unlikely to stimulate a sufficiently high or maintained rise in TSH to promote carcinomata. These patients are likely, however, to develop carcinomata if submitted to a goitrogenic environment or regime at a later date.

The carcinogenic effect of small doses of x irradiation in infants suggests that their thyroid tissue might be more radiosensitive than that of adults. It has also been suggested that loss of thymic function might lead to an increase in circulating TSH or in thyroid response to TSH (Simpson & Hempelmann, 1957). Experiments on weanling rats (Doniach, 1957, 1958) have not supported these hypotheses. However, one need look no further for a promoting factor than the normal growth of the thyroid from infancy to early adult life. The human thyroid grows by mitotic division of its cells from a weight of 2 g. in infancy to 25 g. in the young adult. The situation is thus comparable with goitrogen-induced tumours in rats after 1,100 rads x rays. (Prolonged methylthiouracil also produces a tenfold increase in the weight of the adult rat's thyroid, i.e., from 25 mg. to 250 mg.)

Two points of interest arise from the findings in children. One is the comparative smallness of the dose of radiation which is required to induce potential malignancy, and this may well apply to other tissues. The other is that in this instance the promoting agent is not necessarily TSH. Total dependence of the thyroid in the new-born animal on the pituitary for its initial growth and development has not yet been proved.

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THE DOSE-RESPONSE RELATIONSHIP IN RADIATION CARCINOGENESIS

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1 Human experience
2 Animal experiments
3 Dose-response relations: their value and interpretation
References

A dose-response curve for tumour production must be based on three kinds of information: (i) the number of individuals carrying tumours; (ii) the number of individuals exposed to the carcinogen, which between them give the tumour frequency; and (iii) the dose of the carcinogen. Each of these three things must be known for a number of sufficiently different doses before the nature of the dose-response relation can be appreciated.

1. Human Experience

In clinical work the number of individuals with tumours is usually the most easily obtained information. The dose of the carcinogen, whether it be soot or cigarettes or occupational radiation exposure, is likely to be known only approximately, but commonly the greatest difficulty is to determine the number of individuals at risk. If it is possible to define a population independently of dose or response, an epidemiological survey may be possible, but this has happened only rarely.

It has been known for 30 years that radium absorbed into the body and deposited in bone can cause bone tumours, and a number of individuals with and without tumours have had their body burdens of radium determined (Aub, Evans, Hempelmann & Martland, 1952; Hasterlik, 1956). The frequency of tumours caused at any particular dose-level is, however, impossible to determine because of the bias in the selection of individuals for measurement of body burden of radium, i.e. dose. It often happens that these had been seeking medical help, and the number of asymptomatic persons with the same body burdens is unknown. Several other groups of individuals have recently been found who contain radium either from occupational exposure or because they were given it by doctors. Once a population is defined in this way, an epidemiological survey may be made. We may look forward to the possibility of arriving at a dose-response curve for carcinogenesis caused by radium in man, but the data are not available at present.

All the human evidence on dose-response relations for carcinogenesis by radiation comes from studies of the incidence of leukaemia in two populations of irradiated human beings: the survivors of the atomic bomb explosions at Hiroshima and Nagasaki and the individuals with ankylosing spondylitis who were treated by deep *x*-ray therapy (Court-Brown, 1958). Because this evidence is derived from human beings it must be more relevant to human problems than is evidence from animal experiments, but the more important the evidence the more closely it deserves scrutiny.

When a population is selected for investigation because it has been exposed to a carcinogen, there is always the problem of the control group with zero exposure. As far as radiation is concerned there can never be such a control group because we are all continuously irradiated from conception to the grave, but this background dose may well be disregarded when the dose to the population is relatively very much higher. The control for the Japanese survivors is the data on national mortality for leukaemia which

> . . . is justified only by the fact that the number of deaths attributed to leukaemia among survivors . . . who cannot have received more than very small amounts of radiation, is close to the expected number calculated on the basis of these data (Court-Brown & Doll, 1956).

A similarly adequate control group of unirradiated ankylosing spondylitics is not available (Court-Brown, 1958).

Whatever the susceptibility to " spontaneous " leukaemia of individuals with ankylosing spondylitis, whether the same or different from the average, additional information is needed before we can know whether their susceptibility to radiation-induced leukaemia is the same as that of healthy individuals or not. There is good clinical and experimental evidence that inflamed connective tissue is much more sensitive to the induction of sarcoma by radiation than is normal connective tissue (see Glücksmann, Lamerton & Mayneord, 1957), and the physiological state of the bone-marrow in ankylosing spondylitis may possibly provide a milieu in which leukaemia is more easily induced by radiation than normally.

These arguments merely suggest caution in the application to healthy individuals or national populations of a dose-response curve derived from persons with ankylosing spondylitis. The dose-response curve is certainly true for the spondylitics themselves; the greatest care was taken in the collection of the data and the estimates of dose (Court-Brown & Doll, 1957). Whether the dose-response curve is thought to be linear or not (figs. 1, 2 of Court-Brown, 1958) depends on the emphasis the observer places on the leukaemia incidence after the highest dose. The data in themselves are as compatible with a markedly curvilinear relation as with a linear one, as is probably inevitable, considering the relatively small number of cases of leukaemia and their necessary subdivision into four or five dosage groups.

The dose-response curve derived from the Japanese data is unfortunately much less reliable, because the doses received are so uncertain. The survivors have been classified into dosage groups by distance from the hypocentre[1] and there are many possibilities of error (Court-Brown, 1958). Lewis (1957) obtained a linear dose-response relation for leukaemia incidence from three such dosage groups, but the systematic errors in the estimation of dose are mostly in the same direction and suggest that the relation is really curvilinear. When a population is evenly distributed over the ground, the mean dose per person in an annulus around the hypocentre is less than the " air " dose averaged along a radius crossing the ring. The over-estimate of mean dose due to ignoring this factor is greater the closer to the hypocentre and, while only

[1] An imaginary point on the ground directly beneath a nuclear device exploded at some distance above ground level.—Ed.

small for the group 1,500–1,999 m. from the hypocentre, would be some 30% in the 1,000–1,499 m. group. The mean dose for the high dose group at 0–999 m. was given as 1,300 rem (röntgen equivalents man)[2]: on general grounds this seems impossibly high. Neel & Schull (1956), from whom Lewis derived his dose data, stated that the "maximum dose received" was "in the neighborhood of the equivalent of 600 roentgens of gamma rays." It is reasonable to think that the over-estimate of dose in the high dose group was at least 100%. There was thus a systematic over-estimation of dose, proportionally greater the greater the mean dose, and this can easily convert a really curvilinear dose-response relation into an apparently linear one. The uncertainty of the estimations of dose in the Japanese data has deterred most investigators from drawing anything but the broadest conclusions, and these detailed criticisms of Lewis's analysis would hardly have been worth while if his conclusions had not gained a wide currency as a quantitatively accurate assessment.

As judged from obituary notices, the incidence of leukaemia in American radiologists was several times beyond that expected. The dose received was estimated by Lewis at less than 30 rads per year, but other estimates place it between 5 and 50 times higher (Mitchell, 1948; Evans, 1950). The evidence is clearly too uncertain for quantitative use.

2. Animal Experiments

Any properly designed experiment can provide dose-response curves. There may be practical difficulties in carrying out the experiments, and the reason for wanting quantitative data may not be self-evident. Thus, it is generally accepted that quantitative data on carcinogenesis obtained on animals cannot be directly applied to man, and a direct biochemical, cytological or histogenetic approach to carcinogenesis would seem to be much more rewarding than any study of dose-response relationships. Nevertheless, if the carcinogenic effects of radiation are mediated through the effects of radiation on genetic material, there ought to be a close quantitative agreement between dose-response curves in mammals and man simply because their genetic material is similar physically and chemically, and because, according to some theories at any rate, the action of the radiation on this material should be very largely independent of its environment, i.e., of the particular kind of animal whose cells are being irradiated.

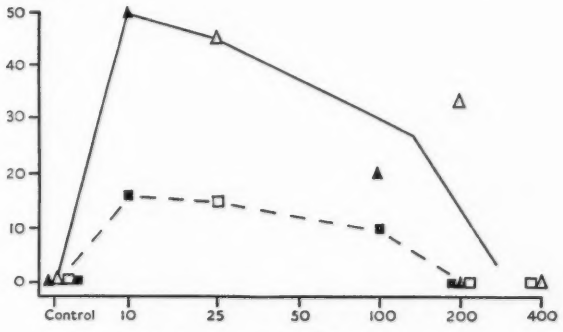
Experimental information, however, is always about the 5–100% range of effect. This is inevitable: hardly any investigator can afford the time and space to have more than 100 individuals in a single dosage group, and this is only just enough to establish with statistical confidence ($P = 0.03$) that a 5% effect is different from a zero response. Thus the range of response in experiments is likely to be different from the clinically important range, which for carcinogenesis is two orders of magnitude less, i.e. 0.05–1%. Often the investigator uses the "optimum" dose in order to keep the number of animals down to a minimum, though this may in fact have the consequence that the data are not usable quantitatively.

Lindsay, Potter & Chaikoff (1957) tabulated the incidence of adenomata and of carcinomata of the thyroid in rats given different doses of ^{131}I. Their data are given in fig. 1 and show a similar dose-response relation for the two kinds of tumour. It is clear that the real shape of the dose-response curve cannot be determined without additional information from doses less than the smallest actually used. Moreover, quantitative comparisons between different carcinogens, say ^{131}I and externally applied *x* radiation, depend on defining the dose-response curve; a dose of one carcinogen in the region where the response is increasing with increasing dose (fig. 1) might otherwise be compared with a dose of the other carcinogen in the region where the response is decreasing with increasing dose. The labour of validating such a comparison is admittedly great, and often the value of the comparison may not justify the labour.

The data of Lindsay *et al.* (1957) given in fig. 1 illustrate another aspect of the relation between dose and effect which is highly relevant to much experimental work on carcinogenesis by radiation. With a large enough dose of carcinogen, no thyroid tumours were observed, because the thyroid remnants were so damaged by the radioiodine as to be unable to respond. When the dose of carcinogen is so large that the responsivity of the tissue is markedly diminished, the results may not be clinically relevant, for in human experience the dose of carcinogen is only very rarely as large as this. As far as radiation is concerned, one major question, in fact, is whether a prerequisite for carcinogenesis is a certain minimum degree of tissue damage and, unless small enough doses are used, this question cannot be investigated. The work on radiation carcinogenesis in the skin discussed by Glücksmann *et al.* (1957) is concerned almost wholly with doses which produced ulceration of the skin. ^{90}Sr produced multiple bone tumours in rabbits, but the doses used also caused gross bone damage (Owen, Sissons & Vaughan, 1957). There can be no refutation *a priori* of the criticism that the mechanism of carcinogenesis after such large, damaging doses is different from that after small doses which do not produce such damaging side-effects.

Many animal experiments suggest that there is a threshold dose and above this a curvilinear dose-response curve; a small

FIG. 1. DOSE-RESPONSE RELATION FOR THYROID TUMOURS IN RATS GIVEN A SINGLE INJECTION OF ^{131}I



Abscissae: dose of ^{131}I (μC)
Ordinates: incidence of tumours (%)

△ adenomata in males
▲ adenomata in females
□ carcinomata in males
■ carcinomata in females

The data are derived from Lindsay, Potter & Chaikoff (1957).

[2] A rem is that absorbed dose in tissue that is considered to result in biological damage equivalent to that produced by one rad of *x* radiation (of about 200 kv).—Ed.

change in dose, say twofold to fivefold, may cause a marked change in tumour incidence from the control level to 15–30% (Table I). For some of the neoplasms listed in the table, e.g., ovarian tumours and lymphoid tumours (leukaemia) in *x*-irradiated mice, and pituitary tumours in mice given ^{131}I, there is good evidence of the importance of influences arising in unirradiated parts of the animal. When this is so, it is perhaps to be expected that tumours should not begin to appear in any numbers until a sufficiently large general disturbance had been produced, and that, once this threshold has been exceeded, the rate of tumour production should increase progressively with increase in dose. The same kind of curvilinearity was found consistently in all the other experiments listed in Table I and it is possible that effects additional to those arising in the locally irradiated areas are equally important for all radiation-induced neoplasms. Alternatively, since the method of selection of experiments for inclusion in the table necessarily entails that the only tumours which are included are those from tissues capable of giving a high tumour frequency, it may be that it is only for tumours of such tissues that influences from distant, unirradiated parts of the anima are important.

Dose-response curves covering a wide range of response have been obtained for leukaemia following whole body irradiation of mice and for bone tumours in mice injected with a variety of radioactive materials. The variation in incidence of leukaemia with dose after a single exposure of LAF_1 mice to an atomic explosion (Furth & Upton, 1954a) is plotted in fig. 2. Thymic and non-thymic leukaemia are distinguished. For thymic leukaemia the dose-response curve is markedly curvilinear, little increase in incidence being observed until the dose approached the level which kills acutely. For non-thymic leukaemia the incidence decreased with dose in a reciprocal manner, and the total incidence of leukaemia was relatively little changed. Similar results were obtained in another strain of mice, the Rfl, given single doses of *x* rays (Furth & Upton, 1954b). One deduction might be that radiation merely changes the way in which the leukaemic process expresses itself without actually causing leukaemia. However, if account is taken of the relation between age of

TABLE I. DOSE AND TUMOUR INCIDENCE IN IRRADIATED ANIMALS

Reference	Source of radiation	Animal	Site of tumour	Unit of dose	Dose giving tumour incidence of		
					0–2% (i.e. no greater than control)	15–30%	50–100%
Deringer, Lorenz & Uphoff (1955)	Whole body *x* rays (single dose)	Mouse	Ovary	r.	25	—	50
Deringer *et al.* (1955)	*x* Rays to ovarian region only (single dose)	Mouse	Ovary	r.	25	50	200
Kaplan & Brown (1952)	Whole body *x* rays (one dose or two doses on successive days)	Mouse	Lymphoid tissue	r.	(283)*	400	—
Furth & Tullis (1956)	^{131}I (single injection)	Mouse (male)	Pituitary	μc.	25	—	50
Lisco, Finkel & Brues (1947)	Oral ^{91}Y for three months	Rat	Colon (adenocarcinoma)	μc./feed	60	—	200–460
Finkel, Lisco & Brues (1955)	^{89}Sr by injection (single dose)	Mouse	Bone	μc./kg.	200	1,000	2,500
Finkel & Scribner (1956)	^{90}Sr by injection (single dose)	Mouse	Bone (malignant)	μc./kg.	9†	200	440
Finkel & Scribner (1956)	^{45}Ca by injection (single dose)	Mouse	Bone (malignant)	μc./kg.	1,100	—	5,600
Koletsky & Christie (1954)	^{32}P by injection (single dose)	Rat	Bone (sarcoma)	μc./kg.	500	1,000	—
Kuzma & Zander (1957)	^{90}Sr by daily injection (ten successive days)	Rat	Bone (malignant)	μc./kg. (total)	100	—	500
Kuzma & Zander (1957)	^{90}Sr by monthly injection (ten successive months)	Rat	Bone (malignant)	μc./kg. (total)	1,000	—	2,500
Kuzma & Zander (1957)	^{45}Ca by daily injection (ten successive days)	Rat	Bone (malignant)	μc./kg. (total)	1,000	2,500	3,500
Kuzma & Zander (1957)	^{45}Ca by monthly injection (ten successive months)	Rat	Bone (malignant)	μc./kg. (total)	2,500	3,500	—

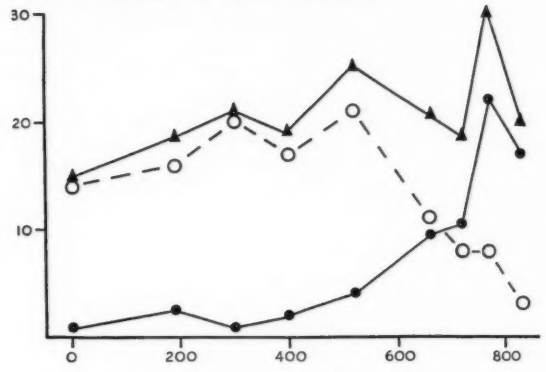
* Control level 6–8%
† The dose of 90 μc. ^{90}Sr/kg. body-weight produced 5% tumours

The data assembled in this table are from all those experiments on radiation carcinogenesis known to me where :

i. the over-all incidence of tumours was determined at two or more dose-levels ;
ii. one dose produced no effect, i.e. no increase above the control incidence;
iii. another dose produced a substantial frequency of tumours (as indicated in the two columns at the extreme right).

Given ii and iii, some idea of the shape of the dose-response curve may be obtained. If the curve were really linear, the dose which produced 15–30% tumours should be at least 7–15 times the dose which produced 2% tumours, and correspondingly greater if the tumour yield was 50% or more. The dose ratios are all less than expected on the linear hypothesis, which shows that the efficiency of tumour production increased with dose. The number of animals in each group was small in most of the experiments but the consequent statistical uncertainty is more than counterbalanced by the consistency with which the different experiments show the same kind of curvilinearity of tumour yield against dose. Similarly shaped curves have been obtained for thymic leukaemia (fig. 2) and for pituitary tumours in mice given a single exposure to an atomic explosion (Upton & Furth, 1955).

FIG. 2. THE INCIDENCE OF LEUKAEMIA IN MALE LAF_1 MICE AFTER EXPOSURE TO RADIATION FROM AN ATOMIC EXPLOSION



Abscissae: radiation dose (r.)
Ordinates: incidence of tumours (%)

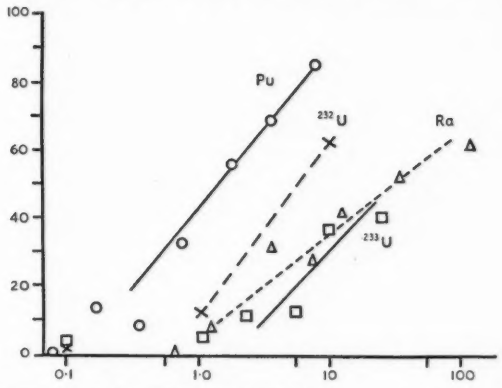
●——● thymic leukaemia
○---○ non-thymic leukaemia
▲——▲ all types of leukaemia

The data are derived from the paper by Furth & Upton (1954a), where the time-course of leukaemia development after irradiation is given. Female mice gave dose-response curves of similar shape, but the over-all incidence in controls was greater than in any irradiated group.

mouse and type of leukaemia (Furth & Upton, 1954b; Mole, 1958), and of the fact that the mean life-span was shortened to a greater degree the larger the dose of radiation, it is possible that non-thymic leukaemia was reduced in incidence as the dose was increased merely because the mice did not live long enough for the diagnosis to be made. This may be another example of damaging side-effects interfering with the expression of malignant disease. Analysis of the data in terms of the probability of developing leukaemia at different times during the life of the mouse might give a quite different dose-response curve.

During the last 15 years, extensive experiments on the production of bone tumours in the mouse by varying doses of a number of different bone-seeking radioisotopes have been carried out at the Argonne National Laboratory, Chicago (Lisco, Finkel & Brues, 1947; Brues, 1949; Finkel, 1953, 1956). During this time methods of animal management and the mortality of controls at different ages have inevitably changed. Comparisons of results obtained several years apart are then better made by considering the tumour expectancy rather than the over-all tumour incidence. Figure 3 shows the total tumour expectancy after single injections of various doses of four different radioactive elements, all α-particle emitters (Finkel, 1953). Where the tumour incidence is 10% or more, there is a linear relation between tumour expectancy and log (dose), but for reasons discussed above the data are insufficient to show the shape of the dose-response curves where the tumour incidence is less than 10%. In earlier work on the effects of monthly injections of ^{89}Sr (fig. 4) there was a systematic dependence on dose of the latent period before any tumour appeared, and of the probability of tumour development. Whether the latent period always depends on dose is still an open and important question. If the latent period

FIG. 3. PROBABILITY OF DEVELOPING A MALIGNANT BONE TUMOUR IN THE MOUSE, AFTER A SINGLE INJECTION OF AN α-EMITTING RADIOISOTOPE



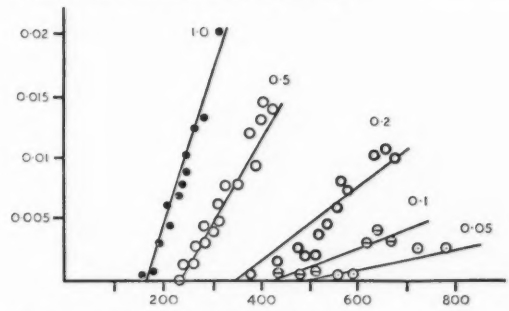
Abscissae: dose of radioisotope (μC/kg.)
Ordinates: percentage of surviving population with malignant bone tumours

The figure is redrawn from Finkel (1953), with the addition of best-fitting straight lines drawn by eye. The method of calculating the probability of developing a tumour is given by her. The control value was 3.3%.

increases as the dose decreases, there must come a point where the latent period is greater than the maximum life-span, and the dose where this occurs is then a true threshold dose producing no detectable effect in the species under consideration.

The experiment just discussed and another experiment with ^{90}Sr (Finkel, Lisco & Brues, 1955) are possibly the only ones in which radiation was continuously administered and a dose-response curve for tumour incidence was obtained. A number of chronic irradiation experiments have been done, using externally applied radiation of a penetrating kind, e.g., γ rays or fast neutrons. In view of the doses accumulated, the tumour incidence has been surprisingly low, and unfortunately dose-response curves are not easily deducible from the results (for discussion see Neary, Munson & Mole, 1957).

FIG. 4. THE DAILY PROBABILITY OF BONE TUMOUR DEVELOPMENT IN THE MOUSE, FOLLOWING MONTHLY INJECTIONS OF ^{89}Sr



Abscissae: time after first injection (days)
Ordinates: daily probability of bone tumour development
Curves show effect of monthly doses of ^{89}Sr, of 1.0, 0.5, 0.2, 0.1, and 0.05 μC/g.

The figure is redrawn from Brues (1949).

3. Dose-Response Relations: their Value and Interpretation

The idea that carcinogens may be weak or strong is ambiguous, but if two carcinogens are to be quantitatively compared, it is necessary to define a dose-response curve for each of them; an estimate of tumour incidence after one dose of each is clearly insufficient. Further, if the difference between the two carcinogens is thought to be merely quantitative and a numerical factor is wanted to describe the difference, it must be shown that the shapes of the dose-response curves are the same when dose is expressed on a logarithmic scale. Only then will the numerical factor be independent of the degree of response.

This kind of information is being sought in the work on the production of bone tumours by α- and β-particle emitters referred to earlier, and numerical factors are needed for the practical purpose of setting safe levels for human exposure. There is human experience of bone tumour production by radium and, however quantitatively limited the evidence may be, the safe level for human exposure to radium does not entirely depend on extrapolation from animal experiments. Human experience of bone tumour production by fissionable material, like plutonium, or fission products, like radioactive strontium, does not exist at present, and in the atomic energy industry every effort is made to prevent such evidence from materializing. Safe levels for working conditions have to be decided, therefore, in the absence of human evidence. To do this the experience with radium has been taken as the baseline, and the safe dose for other materials has been derived from the radium dose by scaling the dose up or down by numerical factors derived from animal experiments.

A practical procedure of this kind need not depend on any hypothesis about the nature of cancer or the mechanism of carcinogenesis. From an academic view-point, however, dose-response curves may be of value in deciding whether two carcinogens have a similar mode of action, or even in deciding between different hypotheses of carcinogenesis. If by somatic mutation is meant point mutation, then the somatic mutation hypothesis of carcinogenesis implies that the yield of tumours will increase linearly with an increase in effective dose, and be independent of the way in which the dose is spread out in time. The " effective dose " of a chemical carcinogen is never easy to define, since its concentration at a tissue waxes and wanes with time. From this point of view radiation may be a better investigative tool because its application in time and space can be more exactly defined. It is clear from the data given in Table I and in figs. 2–4 that " None of the animal experiments have indicated a linear relationship between tumour incidence and dose " (Glücksmann *et al.* 1957).

It is probably too naïve to expect a linear dose-response relation for carcinogenesis by radiation even if the mutation hypothesis were true, for radiation is known to kill cells, and mutated cells will be no exception. The yield of tumours would depend on the net survival of mutated cells, and the dose-response relation might be quite complex, depending on the relative mutagenic and lethal effects of the dose or doses given and the physiological state of the irradiated animal or tissue, i.e., the environment in which the potentially neoplastic cells must multiply before the tumour becomes manifest. The additional evidence of the importance of dose-rate to tumour yield (Mole, 1958), if confirmed, amounts to a formal disproof of the point mutation hypothesis as far as thymic leukaemia after moderately large doses is concerned.

Even if a dose-response curve is purely empirical, it is sometimes thought that it should provide conclusive evidence about the existence or not of a threshold dose below which no response would occur. This seems to me a vain hope. Whatever the shape of the empirically determined curve, it is always possible that the shape of the curve changes at doses less than the lowest used. Even if there were several dose-levels at each of which the response was no different from the base-line control, it must be accepted that each of the measured responses has a statistical error. The possibility can never be denied that observations on a much larger scale would have shown responses really greater than the control. Thus the existence of a threshold can never be proved by an empirical dose-response curve. Equally, of course, for similar reasons the absence of a threshold can never be proved. Thus the existence or not of a threshold can be determined only on theoretical grounds.

Nevertheless it must be recognized that, if an empirical dose-response curve is obtained which suggests some empirical conclusion, and if the conclusion is denied by the use of arguments of the kind outlined above, then there ought to be a scientifically adequate reason for thinking that the nature of the dose-response relation should change at low levels of response. In the field of radiation carcinogenesis this may be provided by the two rival but not mutually exclusive hypotheses, that radiation converts normal cells into potentially neoplastic cells, and that radiation causes tissue damage of which the imperfect repair is responsible for tumour development. From the experimental view-point the crux is whether the change in the nature of the dose-response relation, if it is to be expected, will occur at levels of response which can be investigated experimentally (see section 2).

If carcinogenesis is a multistage process, however, it may not be sensible to think in terms of dose-response curves, for then any particular curve must depend markedly on the conditions under which it was obtained. The relation between dose and leukaemia incidence in Japanese survivors of atomic explosions and in British patients with ankylosing spondylitis is fairly similar. Whether this evidence can be used to indicate how much leukaemia would be produced by an increase in background radiation where the dose-rate is at least a million times less, depends on whether a difference of dose-rate is a relevant parameter. Clinical endeavour, too, is always directed to uncovering the reasons for individual susceptibility, and dose-response curves applicable to a general population lose much of their value if it becomes possible to define subgroups of quite different sensitivities. Nevertheless, however complex the carcinogenic process, the task of defining the quantitative importance of each of the factors involved is a necessary preliminary to efficient prophylaxis.

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Notes on Contributors

DR J. M. BARNES has been Director of the Medical Research Council's Toxicology Research Unit since its inception in 1947. The work of the Unit is devoted to a detailed study of the mode of action of certain toxic substances which find a use in industry or agriculture. Much of Dr Barnes's experimental work has been concerned with the toxic action of insecticides and he is author of a World Health Organization monograph on this subject entitled "Toxic hazards of certain pesticides to man, together with a select bibliography on the toxicology of pesticides in man and mammals" (1953, *Monogr. Ser.* No. 16). His other interests relate to the design of suitable tests for assessing the toxic properties of new materials. Dr Barnes has previously contributed to *British Medical Bulletin* a paper entitled "Health hazards from insecticides" (*Brit. med. Bull.* 1950, **7**, 23).

DR F. BIELSCHOWSKY was Privatdozent at the University of Freiburg and lecturer on metabolic diseases in Madrid before coming to Britain. He worked from 1939 until 1948 in the Department of Pathology of the University of Sheffield. After collaborating with Professor H. N. Green in an investigation of the mode of action of sulphanilamide, he began in 1942 to work on problems of cancer, studying the action and the metabolism of aromatic amines. His earlier work dealt with problems of the metabolism of nucleic acids and of lipids. Dr Bielschowsky has since 1948 been Director of the Cancer Research Department of the Medical School, University of Otago, Dunedin, New Zealand, and of the New Zealand branch of the British Empire Cancer Campaign. During the last ten years he has studied the endocrinological aspects of cancer (see *Brit. J. Cancer*, 1955, **9**, 80). Dr Bielschowsky has previously contributed to the Bulletin a paper on the carcinogenic action of 2-acetylaminofluorene and related compounds (*Brit. med. Bull.* 1947, **4**, 382).

DR G. M. BONSER is Reader in Cancer Research in the University of Leeds and Consultant in Morbid Anatomy, St James's Hospital, Leeds. She has been engaged in cancer research for thirty years, working first on intra-thoracic cancer and later on the hormonal and genetic aspects of mammary and testicular cancer. During the last twenty-five years, she and her colleagues have undertaken a combined biological and chemical investigation of the metabolism and carcinogenic properties of aromatic amines, especially of those related to the dye intermediates. Her publications include: "A microscopical study of the evolution of mouse mammary cancer: the effect of the milk factor and a comparison with the human disease" (*J. Path. Bact.* 1945, **57**, 413); with E. C. Armstrong, "The carcinogenic action of 2-acetylamino-fluorene on various strains of mice" (*J. Path. Bact.* 1947, **59**, 19); and, with L. Bradshaw, D. B. Clayson & J. W. Jull, "A further study of the carcinogenic properties of ortho hydroxy-amines and related compounds by bladder implantation in the mouse" (*Brit. J. Cancer*, 1956, **10**, 539). Dr Bonser contributed a paper to an earlier number of the Bulletin, on experimental cancer of the bladder (*Brit. med. Bull.* 1947, **4**, 379).

PROFESSOR E. BOYLAND is Professor of Biochemistry in the University of London at the Chester Beatty Research Institute, Royal Cancer Hospital, where he has worked (except for the war years) since 1931. He has carried out work on the metabolism of aromatic compounds, the chemotherapy of cancer, the metabolism of tumours, and in the field of carcinogenesis. In work on metabolism of aromatic compounds he discovered the metabolic process of perhydroxylation (e.g., "The conversion of anthracene to 1:2-dihydroxy-1:2-dihydroanthracene" (Boyland & Levi, *Biochem. J.* 1935, **29**, 2679); "The nature of the acid-labile mercapturic acid precursor excreted when animals are treated with naphthalene" (Boyland & Sims, *Biochem. J.* 1958, **68**, 440)). Professor Boyland has also identified 15 metabolites of the carcinogenic 2-naphthylamine (cf. Boyland & Manson, *Biochem. J.* 1957, **67**, 275). In the field of carcinogenesis he demonstrated the carcinogenic activity of dibenzcarbazoles (Boyland & Brues, *Proc. roy. Soc.* B, 1937, **122**, 429), nitrogen mustards (Boyland & Horning, *Brit. J. Cancer*, 1949, **3**, 118), and of the tryptophan metabolite 3-hydroxyanthranilic acid (Allen, Boyland, Dukes, Horning & Watson, *Brit. J. Cancer*, 1957, **11**, 212). During the war Professor Boyland worked for the Ministry of Supply on problems of chemical warfare and for the Ministry of Agriculture on animal diseases. He received the Judd Award for Cancer Research in New York in 1948 and the medal of the Société de Chimie Biologique in 1956. Before starting cancer research he studied in the Department of Physiology of the University of Manchester, the Lister Institute for Preventive Medicine in London, and the Kaiser Wilhelm Institut für medizinische Forschung in Heidelberg. At present he is working on the causes of cancer of the bladder, intestine, lung and cervix, on the treatment of cancer of the bladder and breast, and on the metabolism of aromatic compounds, and he is interested in carcinogenic hazards, particularly in foods, contraceptives, tobacco and cosmetics. Professor Boyland contributed a paper, together with Dr F. Weigert, to an earlier number of the Bulletin, entitled "Metabolism of carcinogenic compounds" (*Brit. med. Bull.* 1947, **4**, 354).

DR W. CARRUTHERS graduated as Ph.D. in chemistry at the University of Glasgow in 1949, then took up an appointment with the Medical Research Council at Glasgow to investigate the chemistry of high-boiling carcinogenic fractions of mineral oils, under the direction of Dr J. W. Cook. In 1956, he moved to Exeter, and took charge of the laboratory of the Council's new Carcinogenic Substances Research Group. The present work of the Group includes a study of the chemistry of tobacco smoke, as well as a continuation of the investigation of mineral oil fractions. A number of papers have been published incorporating the results so far obtained in the mineral oil work.

DR D. B. CLAYSON is Elizabeth and Louisa Ward Fellow in the Department of Experimental Pathology and Cancer Research in the University of Leeds. After training as an organic chemist at Oxford he came to Leeds in 1948 to work with Dr G. M. Bonser on the relation between the metabolic fate of the aromatic amines and their carcinogenic action. His published work includes: with G. M. Bonser & J. W. Jull, "An experimental inquiry into the cause of industrial bladder cancer" (*Lancet*, 1951, **2**, 286); "A working hypothesis for the mode of carcinogenesis of aromatic amines" (*Brit. J. Cancer*, 1953, **7**, 460); and, with G. M. Bonser, L. Bradshaw & J. W. Jull, "A further study of the carcinogenic properties of ortho hydroxy-amines and related compounds by bladder implantation in the mouse" (*Brit. J. Cancer*, 1956, **10**, 539).

DR J. W. COOK is the first Vice-Chancellor of the University of Exeter and is also Honorary Director of the Medical Research Council's Carcinogenic Substances Research Group. In 1939 he was appointed Regius Professor of Chemistry in the University of Glasgow, a post which he held for 15 years. Before that he was a professor of chemistry in the University of London and research chemist to the Royal Cancer Hospital. He was responsible, with I. Hieger and C. L. Hewett, for the isolation from coal-tar pitch of a potent carcinogenic pure hydrocarbon and for demonstrating by synthetic preparation that this was the hitherto unknown 3:4-benzopyrene. He also prepared synthetically many other carcinogenic polycyclic hydrocarbons and was associated with E. C. Dodds and C. L. Hewett in the discovery of the synthetic oestrogens. Dr Cook was awarded the Davy Medal of the Royal Society in 1954 and was the Chemical Society's Pedler Lecturer in 1950. He has been President of the Royal Institute of Chemistry and was President of the Chemistry Section at the 1957 meeting of the British Association for the Advancement of Science.

DR W. M. COURT-BROWN is on the Scientific Staff of the Medical Research Council and is Director of the Group for Research into the General Effects of Radiation. He studied medicine at St Andrew's University where he graduated in 1942. After some years of work in radiotherapy, he commenced in 1950 the study of the harmful effects of ionizing radiations. He spent some time in the investigation of the acute effects, as a result of which a number of papers were published dealing with the factors governing the length of the latent period between exposure to radiation and the onset of gastro-intestinal symptoms, and he has now become mainly interested in the study of the long-term carcinogenic effects. He has published several papers on this subject, and particularly, in co-operation with Dr Richard Doll, a study of the leukaemogenic effects of radiations (*Spec. Rep. Ser. med. Res. Coun., Lond.* 1957, No. 295).

DR I. DONIACH, Senior Lecturer in Morbid Anatomy, Postgraduate Medical School of London, was formerly cancer research assistant to Dr J. C. Mottram at Mount Vernon Hospital, London. His experiments have been mostly concerned with the carcinogenic action of radioactive iodine on the rat's thyroid and with changes in thyroid function resulting from radiation. Publications include: "A comparison of the photodynamic activity of some carcinogenic with non-carcinogenic compounds" (*Brit. J. exp. Path.* 1939, **20**, 227); and "The effect of radioactive iodine alone and in combination with methylthiouracil upon tumour production in the rat's thyroid gland" (*Brit. J. Cancer*, 1953, **7**, 181).

DR L. A. ELSON is Reader in Biochemistry in the University of London. His first research work was on chemotherapy at Guy's Hospital Medical School, London. He was later Jenner Memorial Scholar at the Lister Institute of Preventive Medicine, London. After some years with Imperial Chemical Industries Limited in Manchester, he came to work at the Chester Beatty Research Institute, Royal Cancer Hospital, London, where he held a British Empire Cancer Campaign Fellowship and was appointed Reader in Biochemistry in 1956. Among his publications, one describes the well-known Elson and Morgan method for the determination of glucosamine and chondrosamine (*Biochem. J.* 1933 **27**, 1824). He has contributed a series of papers to the *British Journal of Cancer* on the influence of the protein content of the diet on the growth-inhibitory action of carcinogenic and tumour-inhibitory compounds. At present he is mainly engaged on work on the comparative biological effects of radiation and radiomimetic tumour-inhibitory and anti-leukaemic chemicals.

DR A. GLÜCKSMANN is Senior Histologist to the Strangeways Research Laboratory, Cambridge, which he joined in 1933. He has worked mainly on

the effects of irradiation in producing malignant diseases and the effect of irradiation in the treatment of malignant disease in man. He has developed quantitative histological methods to assess these effects, and built up probably the largest collection of biopsy and operation material obtained from irradiated human cancers. He received the Röntgen Award of the British Institute of Radiology in 1942, and was Douglas Lea Memorial Lecturer to the Hospital Physicists' Association in 1953. Among his publications are: "The influence of systemic factors on the differentiation and radiocurability of cervical cancers" (*Brit. J. Radiol.* 1956, **29**, 483); "Relationships between hormonal changes in pregnancy and the development of 'mixed carcinoma' of the uterine cervix" (*Cancer, N.Y.* 1957, **10**, 831); "Local factors in the histogenesis of hypertrophic scars" (*Brit. J. plast. Surg.* 1951, **4**, 88). Dr Glücksmann also contributed a paper, "Quantitative histological analysis of radiation-effects in human carcinomata", to an earlier number of the Bulletin (*Brit. med. Bull.* 1946, **4**, 26).

Dr M. W. Goldblatt, formerly Director of Industrial Hygiene Research Laboratories of Imperial Chemical Industries Limited, is now engaged at Pollards Wood Research Station (Institute of Cancer Research, Royal Cancer Hospital). He is a member of the Permanent International Commission on Industrial Medicine, Examiner in Industrial Health for the Diploma in Industrial Health, Member of the Editorial Committee of the *British Journal of Industrial Medicine* and Member of Executive of the British Occupational Hygiene Society. He has published papers on physiology, biochemistry, industrial toxicology and carcinogenesis, including one in an earlier number of the Bulletin entitled "Occupational cancer of the bladder" (*Brit. med. Bull.* 1947, **4**, 405). Dr Goldblatt was created C.B.E. in 1955.

Professor H. N. Green is Professor of Experimental Pathology at Leeds University and Director of Cancer Research at Leeds and Sheffield Universities. He is a Sheffield graduate and, after a period as Lecturer in Pathology at Cambridge, he was for a long time Professor of Pathology at Sheffield. He has published numerous papers on many aspects of experimental pathology, including particularly the subjects of nutrition, traumatic shock and cancer. He commanded a British Traumatic Shock Team, Royal Army Medical Corps, in the Second World War. With H. B. Stoner, he wrote the book *Biological actions of the adenine nucleotides* (1950), which was largely an outcome of the shock investigations. These authors contributed previously to this Bulletin an article entitled "Effects of injury on carbohydrate metabolism and energy transformation" (*Brit. med. Bull.* 1954, **10**, 38) in a symposium on "Reactions to Injury". His immunological theory of cancer (*Brit. med. J.* 1954, **2**, 1374) has stimulated work in this field over a wide front.

Professor Alexander Haddow, F.R.S., graduated in medicine in 1929, at Edinburgh, where he became lecturer in bacteriology (1931–36). He joined the staff of the Research Institute of the Royal Cancer Hospital in 1936 and was appointed, in succession to Professor E. L. Kennaway, to the Chair of Experimental Pathology in the University of London and the directorship of the Chester Beatty Research Institute of the Royal Cancer Hospital in 1946. He has published a number of papers on special aspects of growth, such as the comparative pathology of tumours, viruses in relation to the aetiology of cancer, and problems of immunity affecting the transplantation of tissues. One of his main interests has been in bacterial mutation, and its importance in elucidating what he considers to be analogous problems of variation in the origin of cancer. In 1935, Professor Haddow described the growth-inhibitory property of carcinogenic compounds, and he has developed this subject and related it to the mechanism of action of tumour-producing and growth-inhibiting agents. He has been closely concerned with developments in chemotherapy. With his advice, the general plan of the Bulletin symposium published in 1947, "Chemical Carcinogenesis", was devised. He contributed to that symposium three papers, one in collaboration with G. A. R. Kon, on carcinogenesis and chemotherapy of cancer (*Brit. med. Bull.* 1947, **4**, 314, 331, 417). He is at present engaged (with Professor F. Bergel, Dr W. C. J. Ross and Mr G. M. Timmis) in a study of the growth-inhibitory alkylating agents derived from nitrogen mustard.

Dr I. Hieger is a biochemist at the Chester Beatty Research Institute of the Royal Cancer Hospital, London. He was a member of the team of workers which under the inspired leadership of Sir Ernest Kennaway discovered the carcinogenic hydrocarbons and isolated 3 : 4-benzopyrene from pitch (Cook, J. W., Hieger, I., Kennaway, E. L. & Mayneord, W. V., *Proc. Roy. Soc.* B, 1932, **111**, 455). He is now engaged on the investigation of carcinogenic agents of biological origin (*Proc. Roy. Soc.* B, 1957, **147**, 84). Dr Hieger has published a popular book on cancer (*One in six: an outline of the cancer problem*, 1955). He has previously published in the Bulletin a paper entitled "Carcinogenic substances in human tissues" (*Brit. med. Bull.* 1947, **4**, 360).

Professor E. S. Horning is Professor of Experimental Pathology (Histopathology) in the University of London at the Chester Beatty Research Institute. Previous to this, he was Reader in Experimental Pathology and, before joining the staff of the Chester Beatty Research Institute, was a scientific member of the Imperial Cancer Research Fund. Whilst holding a Rockefeller Research Fellowship, he worked at the Kaiser Wilhelm Institut, Berlin-Dahlem, and later at Washington University, St Louis, as well as at other research centres in the USA. Professor Horning was also a Beit Memorial Fellow. When at the University of Melbourne, his earlier researches were on the enzymic function of mitochondria in Protozoa. Since then he has published numerous papers in the field of cellular pathology and is now working on the relationship of hormones to carcinogenesis. Together with Dr H. Burrows, Professor Horning contributed to an earlier number of the Bulletin a paper entitled "Oestrogens and neoplasia" (*Brit. med. Bull.* 1947, **4**, 367).

Dr J. W. Jull is a Saltwell Fellow of the Royal College of Physicians of London, working in the Department of Experimental Pathology and Cancer Research in the University of Leeds. He started working with Dr G. M. Bonser in 1949, and since has been interested both in the carcinogenic action of the aromatic amines and the role of ovarian and pituitary hormones in the aetiology of cancer of the breast in the human and the experimental animal. His publications include: "The induction of tumours of the bladder epithelium in mice by the direct application of a carcinogen" (*Brit. J. Cancer*, 1951, **5**, 328); "The effects of oestrogens and progesterone on the chemical induction of mammary cancer in mice of the *IF* strain" (*J. Path. Bact.* 1954, **68**, 547); and "Hormones as promoting agents in mammary carcinogenesis" (*Acta Un. int. Cancr.* 1956, **12**, 653).

Sir Ernest Kennaway was Demonstrator of Physiology at Guy's Hospital, London, 1908–14, and was elected a Radcliffe Travelling Fellow in 1909. From 1931 to 1946 he held the post of Director of the Research Institute of the Royal Cancer Hospital, London, now the Chester Beatty Research Institute. Since 1921, he had been engaged on research on cancer, especially as regards cholesterol as a carcinogen, and cancer of the human lung and cervix. His published papers include: with N. M. Kennaway, "The relation between the incidence and incubation period of cancer in man" (*Yale J. Biol. Med.* 1944, **17**, 139); "The racial and social incidence of cancer of the uterus" (*Brit. J. Cancer*, 1948, **2**, 177); "The identification of a carcinogenic compound in coal-tar" (*Brit. med. J.* 1955, **2**, 749). He was an Honorary Fellow of New College, Oxford, and was elected a Fellow of the Royal Society in 1934. Sir Ernest died on the 1 January 1958, while this number of the Bulletin was in preparation; a tribute to him, and further information about his life and work, are included in the Introduction to this Bulletin.

Dr A. J. Lindsey is Head of the Department of Chemistry at Sir John Cass College, London. He had a number of years in the fine chemical and scientific instrument industries before taking up his academic career in 1936. From 1940 to 1946 he served in the Royal Air Force Volunteer Reserve. His research interests are electrochemistry, radiochemistry and combustion chemistry. In this latter field, he and his research students have developed methods for the analysis of smokes and other combustion products, and they were the first to demonstrate the presence of polycyclic aromatic hydrocarbons in tobacco smoke. The ubiquity of these hydrocarbons in combustion products, and the carcinogenic nature of some of them, have made these researches of considerable interest; they have been supported by the Department of Scientific and Industrial Research and by the Medical Research Council. This work, which has been reported in about forty original papers, has been co-ordinated with similar projects in other centres, especially with that under the late Sir Ernest Kennaway at St Bartholomew's Hospital, London. Published work includes: with R. L. Cooper, "3 : 4-Benzpyrene and other polycyclic hydrocarbons in cigarette smoke" (*Brit. J. Cancer*, 1955, **9**, 304); with J. A. S. Gilbert, "Polycyclic hydrocarbons in tobacco smoke: pipe smoking experiments" (*Brit. J. Cancer*, 1956, **10**, 646); and, with J. M. Campbell, "Polycyclic hydrocarbons in cigar smoke" (*Brit. J. Cancer*, 1957, **11**, 192).

Dr R. H. Mole has been working at Harwell since the beginning of radiobiological research work there in 1948. His special interest has been in recovery from irradiation and its delayed effects (see "Quantitative observations on recovery from whole body irradiation in mice", *Brit. J. Radiol.* 1956, **29**, 563 and *Brit. J. Radiol.* 1957, **30**, 40; "Shortening of life by chronic irradiation: the experimental facts", *Nature*, 1957, **180**, 456; and, in collaboration with G. J. Neary & R. J. Munson, *Chronic radiation hazards. An experimental study with fast neutrons*, 1957). Before this, as graduate assistant in the Nuffield Departments of Clinical Medicine and Pathology, Radcliffe Infirmary, Oxford, Dr Mole had an orthodox training in medicine. From 1944 to 1946 he was a pathologist in the Royal Air Force, and in 1947 Clinical Pathologist, Royal Infirmary, Liverpool.

Professor J. W. Orr is Professor of Pathology and Director of Cancer Research in the University of Birmingham, and Honorary Consultant Pathologist to the United Birmingham Hospitals. He is a graduate of the Queen's University of Belfast, and has held the posts of First Assistant Pathologist at St Mary's Hospital, Paddington, London, and Reader in Experimental Pathology in the University

NOTES ON CONTRIBUTORS

of Leeds. His research work has been mainly concerned with problems of experimental carcinogenesis in the skin, breast, liver, ovary and connective tissue, and with the pathology of human cancer in man. His publications include: "The production of liver tumours by azo compounds" (*Brit. med. Bull.* 1947, **4**, 385); "The chemical induction of mammary and ovarian tumours" (*Acta Un. int. Cancr.* 1956, **12**, 682); and, with Dr J. G. Jackson, "The ducts of carcinomatous breasts, with particular reference to connective-tissue changes" (*J. Path. Bact.* 1957, **74**, 265).

DR P. R. PEACOCK is Director of the Cancer Research Department of the Royal Beatson Memorial Hospital, Glasgow. He was trained at Middlesex Hospital, London, where, after qualifying in 1924, he held resident appointments and afterwards research scholarships in the Barnato Joel Laboratories until 1928. His early work was mainly concerned with fat-soluble vitamins and with experimental radiology. He was appointed to his present post in 1928, and in 1933 became the Administrative Medical Officer at the same hospital. He was appointed Honorary Lecturer in Pathology to the University of Glasgow in 1943, and elected a Fellow of the Royal Faculty of Physicians and Surgeons of Glasgow in 1944. He was appointed Honorary Treasurer, International Union against Cancer, in 1952. Dr Peacock's published work includes comparative studies of fowl sarcomata induced by chemical carcinogens and of those spontaneous tumours that can be propagated by cell-free extracts (*Amer. J. Cancer*, 1935, **25**, 1; *Brit. J. exp. Path.* 1945, **26**, 357; *Cancer Res.* 1946, **6**, 311). He has made a study of the metabolism of chemical carcinogens and described their natural routes of excretion (*Brit. J. exp. Path.* 1938, **19**, 315, 434; *Brit. J. exp. Path.* 1940, **21**, 227; *Biochem. J.* 1941, **31**, 1276). A paper by Dr Peacock, on the carcinogenic action of heated fats and lipids, was published in *British Medical Bulletin*, 1947, **4**, 364. He is particularly interested in dietary factors related to cancer of the alimentary tract. He holds the view that much human cancer could be avoided, and that closer co-ordination of clinical, statistical and experimental research may lead to identification of some avoidable forms of cancer.

DR M. H. SALAMAN was trained at Cambridge and the London Hospital. After qualification he studied the antigenic structure of vaccinia under Professor S. P. Bedson and later Sir John Ledingham. In 1939 he entered the Cancer Research Department of St Bartholomew's Hospital, and except for three years as a pathologist in the Royal Army Medical Corps, during which he studied serum hepatitis and was able to recommend measures for its prevention, he has continued to work on cancer. He came to the London Hospital in 1946, and was appointed Director of the Cancer Research Department there in 1948, a post which he still holds. He has been mainly interested in the mechanism of carcinogenesis and particularly in the action of "incomplete carcinogens", i.e., agents which can bring about part but not the whole of the process of tumour production. Three representative publications are: "The combining properties of vaccinia virus with the antibodies demonstrable in anti-vaccinial serum" (*Brit. J. exp. Path.* 1937, **18**, 245); with D. E. Lea, "Experiments on the inactivation of bacteriophage by radiations, and their bearing on the nature of bacteriophage" (*Proc. Roy. Soc.* B, 1946, **133**, 434); with F. J. C. Roe, "Incomplete carcinogens: ethyl carbamate (urethane) as an initiator of skin tumour formation in the mouse" (*Brit. J. Cancer*, 1953, **7**, 472).

DR REGINA SCHOENTAL is a member of the Scientific Staff of the Medical Research Council's Toxicology Research Unit at Carshalton, Surrey, England. Before this she had worked on behalf of the British Empire Cancer Campaign at the Sir William Dunn School of Pathology, Oxford; at the Chemistry Department, University of Glasgow; and at the Cancer Research Department, Royal Beatson Memorial Hospital, Glasgow—with the exception of a year spent in the USA (1952-53). She has studied, among other problems, the carcinogenic constituents of coal-tar and shale-oil, and the metabolic and chemical oxidation of polycyclic aromatic hydrocarbons, and she has synthesized some of their phenolic metabolites. Since the discovery of the carcinogenic activity of pyrrolizidine alkaloids for the rat liver, Dr Schoental has been mainly interested in the mechanism of their action, and in the role medicinal plants containing these alkaloids may play in the causation of liver disorders in some parts of the world. Her published work includes: "The oxidation of aromatic rings by purely chemical means" (*Symp. biochem. Soc.* 1950, **5**, 3); "Kwashiorkor-like syndrome and other pathological changes in rats as a result of feeding with *Senecio* alkaloids (isatidine)" (*Voeding*, 1955, **16**, 268); and, with J. W. Cook & E. Duffy, "Primary liver tumours in rats following feeding with alkaloids of *Senecio Jacobaea*" (*Brit. J. Cancer*, 1950, **4**, 405).

DR A. L. WALPOLE is a graduate of London University and a member of the research staff of Imperial Chemical Industries Limited (Pharmaceuticals Division). Since 1942 his chief work with that firm has been concerned with cancer chemotherapy and chemical carcinogenesis. With other members of a research team he discovered tumour-inhibitory properties in alkylating agents containing methylolamide, epoxide and ethyleneimine groups and later investigated the carcinogenic potentialities of compounds of this type. These researches are described in a series of papers written in collaboration with J. A. Hendry, R. F. Homer, D. C. Roberts & F. L. Rose: "Cytotoxic agents: I, Methylolamides with tumour inhibitory activity, and related inactive compounds; II, Bis-epoxides and related compounds; III, Derivatives of ethyleneimine; IV, The carcinogenic actions of some monofunctional ethyleneimine derivatives" (*Brit. J. Pharmacol.* 1951, **6**, 201, 235, 357; 1954, **9**, 306). With M. H. C. Williams & D. C. Roberts, Dr Walpole first described the carcinogenic activity of 4-aminodiphenyl (*Brit. J. industr. Med.* 1952, **9**, 255). He is currently engaged in a systematic investigation of the carcinogenic properties of aromatic amines derived from diphenyl and stilbene respectively.

DR M. H. C. WILLIAMS is the Division Medical Officer to the Dyestuffs and Pharmaceuticals Division of Imperial Chemical Industries Limited, which he joined in 1948. Before taking up this post he was working with the Medical Research Council's Pneumoconiosis Research Unit in South Wales. For eight years, with his colleague Dr A. L. Walpole, he has been carrying out a systematic study of the carcinogenic effects of substituted aromatic amines. His publications include: a paper on the discovery, with Dr Walpole, of the carcinogenic effect of 4-aminodiphenyl (xenylamine) ("Tumours of the urinary bladder in dogs after ingestion of 4-aminodiphenyl", with A. L. Walpole & D. C. Roberts, *Brit. J. industr. Med.* 1954, **11**, 105); with T. S. Scott, "The control of industrial bladder tumours" (*Brit. J. industr. Med.* 1957, **14**, 150); and a chapter on occupational tumours of the bladder in the forthcoming volume III of *Cancer*, edited by R. W. Raven.

DR DENNIS LEYTON WOODHOUSE graduated in chemistry at the University of Birmingham in 1922; he obtained his M.Sc. in 1923. From 1924 to 1930 he held the appointment as Sir Charles Hyde Research Fellow to the Joint Board for Research in Mental Diseases, City and University of Birmingham. He took his Ph.D. in 1930. Dr Woodhouse secured the post of Research Officer to the Birmingham Branch of the British Empire Cancer Campaign in 1931. He was elected a Fellow of the Royal Institute of Chemistry in 1947, and has been Reader in Chemical Pathology (Cancer Research) in the Department of Pathology, Medical School, Birmingham, since 1954. He has published papers, in the *Biochemical Journal*, *Cancer Research*, *British Journal of Cancer*, *Journal of Pathology and Bacteriology*, on chemotherapy of cancer and aspects of carcinogenesis.

Book Reviews

Cancer. Volume 1: Research into Causation

Edited by *Ronald W. Raven*. London: Butterworth & Co. (Publishers) Ltd., 1957. x + 539 pages + 19 index pages; 94 figures. 25 × 17 cm. £4 5s.

The distinguished authors of the twelve essays which compose this volume have each presented an account of a large subdivision of the field. Those who know their way about the special literature may find fuller and more critical accounts of some of the subjects. The virtue of this volume is its broad scope: so much up-to-date information on so many aspects of cancer research has not been gathered in one place before.

Each section gives some account of the early development of the subject, but then passes on to consider mainly the work of the past 20 years. The separate chapter (I) on historical aspects is too short to be of much value to those interested, especially as some of the early literature mentioned is excluded from the reference list. I hope that a further attempt to deal with early ideas and observations will be made in a later volume. For instance, more detailed investigation of the views of those who framed the now well-known questionnaire on cancer published in the *Edinburgh Medical and Surgical Journal* of 1806 would be valuable.

Sir Ernest Kennaway's selection (chapter II) of data on the "incubation period" (a curious variant of the commoner "latent" period) is as stimulating as his writing always was. His argument (chapter III)—that cholesterol may be an important carcinogen because, though feeble, it is ubiquitous—might be applied to other substances.

A specialist is generally dissatisfied with any review which includes his own subject, and it is not surprising that I noticed omissions, and (as I thought) faults of emphasis, in chapter IV, "Carcinogenesis", by P. R. Peacock. I found many provocative ideas in it, however. It is strange that, after so much work on detection of the metabolites of benzopyrene, their role in carcinogenesis has been so little studied. The techniques of initiation and promotion might be useful here. The possibility, well documented in this chapter, that cells are susceptible to carcinogens only during a transitory phase, is one that may repay further thought. That damage followed by regeneration is always necessary is not proved. Initiation of the carcinogenic process, at any rate, can occur without it.

Chapter V, "Chemical mechanisms of normal and abnormal cell division", by J. N. Davidson, is admirable; selective and concise, yet coherent, it covers ground unfamiliar to many. Chapter VI, "Biochemistry of cancer induction", by A. Clark Griffin, brings together a mass of information from various sources. The stress laid on the need for histological control of biochemical studies on tissue changes is admirable, especially coming from a biochemist. I should have appreciated a more critical account of the reputed specificity of combination between carcinogens and cell components.

Chapter VII, "Hormones and neoplasia", by M. B. Shimkin, is a very condensed account of a large and fascinating field of study, which inevitably raises more questions than it answers. An excellent introduction to a subject of great interest to clinicians and laboratory workers! In chapter VIII, "The part played by viruses in the origin of tumours", L. Dmochowski has attempted a task of compression which is perhaps impossible. Yet this is the best up-to-date review of an important but exasperating subject.

Chapter IX, "Nutrition and the genesis of tumours", by A. Tannenbaum & H. Silverstone, is a model of its kind. Not often do men who have devoted a large part of their life's work to a subject succeed in giving such a balanced account of it. Chapter X, "The genetic component of cancer", by P. C. Koller, will be valuable to many who, like myself, lack the basic knowledge necessary to assess the evidence. Chapter XI, "Environmental factors in the production of human cancer", by S. C. Hueper, is the presentation of his subject by an enthusiast. The evidence for the role of the environment can be given several interpretations, but one is left with the impression that industrial contaminants, food additives, atmospheric pollutants, etc., probably have a lot to answer for. Unfortunately, except in a few well-known cases, the evidence is not of the kind on which practical preventive measures could be based. We are faced with the alternatives of drawing uncertain conclusions from human statistics, and relying on animal experiments which may or may not reflect accurately the state of affairs in man. Chapter XII, "Carcinogenic effects of radiation", by A. Glücksmann, L. F. Lamerton & W. V. Mayneord, is one of the most tantalizing as well as interesting of these essays. Apparently so precise a tool, yet how little is known of the way it produces tumours! The most important conclusion drawn is that radiation carcinogenesis is always associated with a severe disorganization of tissue. Whether a specific cellular event is essential is doubtful: the evidence is against it in some cases (e.g. radiation-induced thymoma).

The book will appeal to a wide range of interested people. The standard maintained promises well for the future volumes and supplements. The first volume stands alone, and is likely to prove a useful guide to present knowledge for some time to come.

M. H. Salaman

Cancer. Volume 2: Pathology of Malignant Tumours

Edited by *Ronald W. Raven*. London: Butterworth & Co (Publishers) Ltd., 1958. ix + 641 pages + 25 index pages; 357 figures. 25 × 17 cm. £4 5s.

This volume consists of 33 chapters, each a monograph by a specialist on the malignant tumours of a particular organ, region, or tissue. Only tumours of man are considered here, but spontaneous and induced tumours of animals will be dealt with in the next volume. Benign lesions are mentioned only in so far as they are relevant to corresponding malignant ones.

The scope of the volume is the same as that of R. A. Willis's *Pathology of tumours*. It includes a wealth of up-to-date information and references, many of which have become available since the latest edition (1953) of Willis's book. On the other hand many of the references to older work, and the description of personally collected cases found in Willis, are omitted.

The editor is to be congratulated on achieving sufficient uniformity to make the volume readable, without apparently suppressing the individual style of the contributions.

The field covered is undoubtedly a specialized one, and its value to an individual reader increases directly with his knowledge of general pathology. In view of this the space (57 pages) devoted to introductory matter is excessive. The chapter by L. Foulds (chapter II), since it is theoretical rather than descriptive, would have been better placed in volume 1. Chapters I and III will be of value only to the reader with little background knowledge of pathology.

The authors are predominantly from Britain or the Commonwealth. Nevertheless, a chapter on tumours of the central nervous system has been contributed by James W. Kernohan & George P. Sayre of the Mayo Clinic who, though outstanding in the field, use a classification by no means generally accepted on this side of the Atlantic. In general, the choice of authors is admirable; for example the sections by Cuthbert E. Dukes on the colon, Norman Ashton on the eye, H. A. Sissons on bone and cartilage, and L. M. Franks on the prostate, are excellent. A separate chapter on the heart and blood vessels is welcome, and John Wingrave Landells' contribution on this subject is clearly written and helpful.

The illustrations are generally of good quality. For the morbid anatomist certain tumours present particular difficulties in diagnosis (e.g. chondrosarcoma and mesothelioma), and these might have been more fully illustrated at the expense of some of the commoner tumours. It is to be hoped that one of the promised supplements will include further consideration of these difficult tumours, with ample illustrations.

It is impossible at present to judge the volume as part of the larger work, but by itself it is a considerable undertaking. It has brought together up-to-date views of selected authorities. It has undoubted value as a book of general reference for workers in a number of fields related fairly closely to morbid anatomy, but morbid anatomists themselves will tend to refer to more detailed accounts (in many cases to longer works by contributors to the present volume).

F. J. C. Roe

Biological Aspects of Cancer

Julian Huxley. London: George Allen & Unwin Ltd., 1958. 156 pages; 8 tables. 22 × 14 cm. 16s.

We have to thank Dr Rhoads for inviting Sir Julian Huxley to give the Alfred P. Sloan Lecture which formed the basis of this review.

Having undertaken to have a closer look at the cancer problem, Huxley tackled the job in characteristic fashion and found that it involved him " . . . in more hard labour than anything I have attempted since I took my Final Honours examination in Zoology at Oxford". Faced with the familiar difficulty of not seeing the wood for the trees he proceeds to identify and catalogue the trees, commenting shrewdly, as he proceeds, on their grouping and relative importance. His investigations led to discussions with many prominent cancer research workers and one can imagine that he must have imparted as much as he learned in such exchanges.

The subject is treated in two main sections: A. Comparative and Genetic, which ranges over the whole field of growth (often involving reference to plant and invertebrate life); B. Viral and Epigenetic, which covers the more familiar ground of experimentally induced and propagated tumours, recent virus research, and theoretical discussion.

In the space of 131 pages Huxley covers an enormous amount of work, commenting, as he goes, on useful clues that may appeal to many of his readers. For instance, he seems surprised that more work has not been done with amphibia, in view of the large amount of information about their normal metamorphosis and their capacity for regeneration after injury. Almost every page contains suggestions for further work, some along refreshingly new lines.

It is interesting to learn (p. 49) that the pupation hormone of blow-flies inhibits the carcinogenic action of methylcholanthrene on mice. Sometimes one feels that the headmaster is correcting essays by the top form; "This is irrelevant", he notes in the margin, or "This is an idea worth developing".

Huxley's analysis of the bearing of viruses on tumorigenesis is very timely and avoids over-simplification or dogmatism. How do viruses work and how did they arise? Is a completely saprophytic virus which causes no symptoms to be regarded as a normal cell component under such conditions? Such ideas occur to one as the evidence is surveyed. In considering the nature of some self-replicating virus-like cell constituents, Huxley coins the term "paragene" which many will welcome as more descriptive than "plasmid" or "viroid".

On the practical issue of prevention of cancer, based on available evidence, Huxley is outspoken (p. 118): "It is intellectually pitiful" that the evidence relating cigarette smoking and lung cancer should be dismissed by some critics as only "'the opinion of some statisticians'".

The book ends with a summary comprising 47 sections followed by more than 500 references, many of which are familiar but many quite new to the reviewer. The style of writing and the choice and economy of words are what one would expect from this author.

P. R. Peacock

Leukaemia and Aplastic Anaemia in Patients Irradiated for Ankylosing Spondylitis

W. M. Court-Brown & R. Doll. London: Her Majesty's Stationery Office, 1957. (Medical Research Council Special Report Series No. 295.) vi + 135 pages; figures. 24 × 15 cm. 10s. 6d.

Faced with the fact that the incidence of leukaemia in the United Kingdom had increased from 17 per million in 1931 to 49 per million in 1954, the Medical Research Council set up a Working Party to consider possible aetiological factors. This Special Report concerns an investigation into the suspected increase in leukaemia in irradiated cases of ankylosing spondylitis. Ionizing radiations have been known to be carcinogenic and leukaemogenic in animals and man for many years, but at this time, when the hazards of radiations are becoming a national concern, any clear-cut demonstration of an avoidable hazard is a commendable contribution to medical science.

Court-Brown and Doll, supported by a large team of field workers, examined the records of 13,352 patients irradiated at 81 radiotherapy centres. Their report is in two parts: (i) concerning the incidence of leukaemia and aplastic anaemia in these patients, and (ii) an assessment of the dose-response relationship.

With a latent period of three to five years after irradiation, 46 patients were recorded as having died of leukaemia, aplastic or hypoplastic anaemia, or myelofibrosis, by 31 December 1955. A further three subjects of known leukaemia were still alive at that date. Though 28 patients had died of leukaemia, and 12 of aplastic anaemia, only 2.9 and 0.3 deaths respectively would have been expected to occur in an unirradiated population by that time. The increase in mortality from leukaemia and aplastic anaemia in irradiated spondylitics is therefore highly significant. Reference is also made to an admittedly limited group of unirradiated cases in whom the incidence of leukaemia has been found to be closely similar to that of the general population.

The more important section of this report deals with the assessment of dose-response relationships, and the authors are to be congratulated on their pertinacity in analysing such vast numbers of records, many of which were frustratingly incomplete. Nevertheless they were able to draw some fairly firm conclusions. Having devised several acceptable methods of stating dosage, they then constructed graphs of leukaemia incidence against dose of x rays. They sought to demonstrate either a threshold level of dosage or a proportional relationship indicating a risk at any dose-level. The authors discuss in convincing detail why they feel that a threshold dose is unlikely, and why they hold that the relationship between the dose of radiation and the induction of leukaemia is a simple proportional one, analogous to that seen in the induction of gene mutations.

It seems reasonable to infer from this work that x-ray therapy will have its optimal value and minimal risk if its use is restricted to cases of typical ankylosing spondylitis, in very low dosage, and then only when conservative measures have failed to control severe and diffuse pain. This report is of practical importance to radiotherapists, rheumatologists and orthopaedic surgeons, and extensions of this work will be awaited with interest.

E. C. Easson

Cancer Statistics for England and Wales 1901–1955: A Summary of Data Relating to Mortality and Morbidity

Alan McKenzie with Robert A. M. Case & Joan T. Pearson. London: Her Majesty's Stationery Office, 1957. (General Register Office: Studies on Medical and Population Subjects, No. 13.) iv + 99 pages. 24 × 15 cm. 5s. 6d.

Much of our knowledge of the community aspects of cancer has come from the successive annual and other reports of the Registrar General in which the interesting findings of diverse analyses are reported on. The present volume performs the considerable service of bringing together for easy reference some of the most important national data on the subject in England and Wales during the years of the present century.

Five principal tables deal successively with (i) the changes for each sex in rates of mortality for the major sites over the period 1901–54, (ii) the age and sex differences for selected sites over the years 1936–54, (iii & iv) the standardized death-rates for 1950–54 by sex, region, conurbations and urban and rural aggregates in the selected sites—oesophagus, stomach, intestines and rectum, larynx, lung and bronchus, breast and cervix uteri, and (v) the comparative mortality index (England and Wales) and the standardized mortality rates for standard regions by sex and selected sites over the years 1946–55. Based upon data from the National Cancer Registration Scheme, information is collected on the five-year crude and corrected survival rates by site, sex and clinical stage of disease at registration. In an appendix extensive data are presented showing, for each sex in five-yearly age-groups up to 85 years and over, the death-rates in quinquennial periods from 1901–05 to 1951–55 for malignant diseases of all important sites. In all these tables where changes of classification might disturb the secular trends of the rates, appropriate adjustments have been made to conform with the latest International Statistical Classification.

An introductory text of some ten pages indicates the salient points derivable from the tabular material—the differential sex trends of

mortality from all sites, the considerable reduction in mortality particularly of accessible sites (skin, lip, tongue and mouth, bones, uterus), the recent improvement even in such inaccessible sites as oesophagus in males and stomach and intestine in females, the comparatively static rates for breast cancer and the relentless increase in malignant disease of lung and bronchus and, in less measure, of pancreas, kidney and suprarenal and in leukaemia. Differences in urban and rural incidence are most obvious in lung cancer but are also features in less measure of other sites, but for cancer of the stomach the differences tend to be geographical rather than associates of urban conditions. The information from the Registration Scheme indicates the wide differences of prospects of survival in patients treated in the early stages, the disappointing proportion of early to late cases in accessible sites, such as breast in females, and tongue and mouth in males; the higher proportion of early cases in accessible rather than in the deeper sites; and the very considerable differences in prognosis by site, e.g. from 70% or more survivors in cases of lip, skin and rodent ulcer, to less than 10% in oesophagus, stomach and lung.

The concentration of these extensive statistical data has resulted in an important source of reference to the facts of the cancer situation.

P. L. McKinlay

General Pathology

Edited by *Howard Florey*. 2nd ed. London: Lloyd-Luke (Medical Books) Ltd., 1958. (Based on Lectures delivered at the Sir William Dunn School of Pathology, University of Oxford.) xv + 932 pages; illustrations. 24 × 16 cm. £4 4s.

This well-known series of articles on divers subjects within the scope of general pathology is particularly welcome as giving an authoritative and up-to-date account of the growing edges of pathological science in many directions. The contributions are almost exclusively representative of the British school and to a major extent of that of Oxford. The problems of tumour growth, which are especially relevant to this number of the *British Medical Bulletin*, have been treated by Dr I. Berenblum, now head of the Department of Experimental Biology, the Weizmann Institute of Science, Israel, who contributes a comprehensive review of neoplasia, covering the nature of tumour growth, the epidemiology of cancer, the study of tumours in animals and the classification and morphology of tumours. To the general pathologist the first three of these subjects are of the greatest interest, since "Madame How and Lady Why" are always more intriguing than "Mrs What". Broad conclusions can only be few and these mainly negative: " . . . no basic enzymic defect has so far been demonstrated in neoplasia", and little real knowledge has been acquired of the intimate causes of the cell's "autonomy" (Berenblum dissects and rejects the expression!) in spite of changes in nomenclature and the advent of finer and more penetrating analytical techniques.

The chapter on the epidemiology of cancer includes much wise criticism on the statistics of cancer incidence and goes on to deal with occupational cancers, geographical pathology, and hereditary factors in human cancer. Perhaps the best chapter of the four is that on tumours in animals, which is a close and masterly survey of this wide and involved field. There is an admirable discussion of the influence of genetics, hormones, and the milk factor in carcinoma of the breast in mice, which vividly illustrates the difficulties and perplexing interplay of factors in this single limited field, into which so much time and energy has gone. The section dealing with chemical carcinogens is very detailed and the author points out how their discovery has provided the biologist with better tools for his experimental research and the organic chemist with means of exploring the relationship between chemical structure and carcinogenic activity—a possibility yet to be realized. Perhaps the most suggestive part of this subject is the emergence of the two-stage hypothesis. In this complex of interacting factors how much is fundamental and how much accessory? The next big step seems only round the corner and when it comes the present confusing array of details may assume quite different values from those they present today. Florey's book is presumably for the advanced medical student. Every general pathologist can read it with profit, and to anyone seeking a broad survey of neoplasia, with a balanced summary of recent work, Berenblum's chapters will give the answer.

J. Henry Dible

Essentials of Chemical Pathology

D. N. Baron. London: The English Universities Press Ltd., 1957. xii + 247 pages; 28 figures. 22 × 14 cm. £1 5s.

With the increasing developments in the field of chemical pathology there is necessarily a greater emphasis on the subject in undergraduate teaching. In the reviewer's opinion such teaching should fulfil three requirements: the student should not be overburdened, a requirement which can be aided by the integration of teaching with that in other departments of pathology and in general medicine; the student must be acquainted with the current useful application of the subject to his medical and surgical problems as a doctor; a survey of developing ideas, even if of less immediate practical importance, in relation to the concept of biochemical lesions in the aetiology of disease and its possible application to problems of therapy should be made in order to condition the student's mind to the understanding of future advances in the subject.

Dr Baron has presented his book with a title suggesting that the undergraduate medical student might find it acceptable as a text. This is confirmed in the preface, where the author writes that it has also been designed for medical readers of more senior status. The undergraduate particularly, however, is fortunate to have the book at his disposal as a text. The book is very readable and commendably brief. It broadly fulfils the two requirements of the reviewer which it might be expected to fulfil. It is always possible to disagree with some of the statements made in a dogmatic text, as the views of an individual are necessarily derived from his own experience. In the present case such disagreement may be regarded as the least possible.

In the writing of such a text the problem is always posed of how to present the subject. It is not too safe to assume much recollection of pre-clinical biochemistry by the clinical student and there is a temptation to adopt a biochemical classification under the headings of carbohydrates, lipids, etc. On the other hand it may appear to the student that a more directly practical classification under the headings of body systems, or diseases, would be more useful. Dr Baron has selected an admirable balance according to both these points of view. The result is a useful and well-balanced presentation of the subject, and the practical aspects have not been overlooked. There is a useful table of the composition of normal blood and urine. Rightly the author emphasizes that interpretation of abnormal values can be made only with a full knowledge of the clinical picture and not from tables of diseases (which are omitted) in which any particular abnormality may occur. An appendix presents the practical details of the routine urine tests which need to be familiar to all. To some students the questions quoted from final examinations will be a useful guide to the knowledge expected of them. There is no detailed bibliography to cause confusion, but a list of seven standard works on the subject is appended. Finally, the reviewer found that the index was of the value which indexes should be.

F. T. G. Prunty

Atomic Energy in Medicine

K. E. Halnan. London: Butterworths Scientific Publications, 1957. ix + 157 pages; 14 plates; 31 figures. 22 × 14 cm. 15s.

The man in the street not infrequently wishes to better himself not only financially but in general knowledge. Hence the *Reader's Digest*; hence also such little books as *Atomic energy in medicine*. "This book has been written for readers not necessarily having much knowledge of either physics or medicine . . ." Its importance to physicists and physicians is that it can safely be recommended by them to those earnest laymen who wish to know something of modern progress in this particular field.

The author, Dr K. E. Halnan, is a young physician with experience in radiotherapy and in fundamental scientific research. He writes clearly and entertainingly. Having taken his reader by logical stages through the barest minimum of atomic physics to permit understanding of the principles of nuclear reactors and radioactive isotopes, he introduces him to the use of isotopes in medical research. A short exposition of radiobiology follows as a basis for the second *pièce de résistance*—the place of radiotherapy in cancer. Special chapters are devoted to the use of radioactive isotopes as internally administered treatment for diseases of the thyroid gland and of the blood-forming tissues. And above all

there is a discussion of the losses as well as the profits—the hazards of radiation both to the individual and the race.

This little book is sane and sound and can be read with profit by all.

J. F. Loutit

An Introduction to Medical Laboratory Technology

F. J. Baker, R. E. Silverton & Eveline D. Luckcock. 2nd ed. London: Butterworth & Co. (Publishers) Ltd., 1957. xiv + 408 pages; 118 figures; 14 tables. 22 × 13 cm. £1 15s.

While the first edition established itself as a very valuable textbook for the student technician, the second edition includes added sections on blood transfusion and physiology and new chapters dealing with faeces, gastric analyses and culture media. It is essential for those who intend sitting the Institute of Medical Laboratory Technology examinations.

The new section dealing with blood transfusion deals with the subject by stages, from the preparation of the donor to the collection and storage of the blood. There are also descriptions of the preparation, storage and reconstitution of freeze-dried plasma; and of blood groups and the various blood-grouping techniques, solutions and apparatus used in blood transfusion. The section finishes with the principles of refrigeration. In the section on physiology the authors succeed in compressing a great amount of information into the minimum of space and one feels that this section could be enlarged with advantage in future editions.

There are, however, some minor criticisms which must be made. In dealing with occult blood (chapter 9), the authors would have done well to have added an alternative to the benzidine method of examination which over the past few years has lost favour.

In "Haematological techniques" (chapter 26, p. 275) the suggestion is made that the "pricker" be cleansed with alcohol after each bleeding. This is not enough, as there is a possibility of the transference of serum jaundice, and the authors should have stressed the importance of adequate sterilization of the "pricker" after each bleeding.

In making blood films (p. 276), it is necessary to push the spreader fairly quickly along the slide, not slowly as is stated.

On p. 362 there is a list, "Books consulted and recommended for further reading". The authors should perhaps consult *Microanalysis in medical biochemistry*, by E. J. King & I. D. P. Wootton, 3rd ed.; they will find (p. 32) that under international agreement the British Standard Haldane tube is equivalent to a blood containing 14.6 g. of haemoglobin per 100 ml., and not 14.8 g. In the calculation on p. 290, therefore, "14.8 g./100 ml." should read "14.6 g./100 ml."

The beginner in laboratory work will find this book full of information, set out in a manner that is easy to follow and understand, while the experienced technician will use it as a valuable reference book.

W. R. Weedon

A Dictionary of Scientific Terms

I. F. Henderson & W. D. Henderson. 6th ed., by *J. Kenneth.* Edinburgh: Oliver and Boyd, 1957. xvi + 512 pages. 23 × 15 cm. £1 12s.

The dates of publication of previous editions of this work, which first appeared in 1920, afford an excellent illustration of the acceleration in biological progress which has taken place in the last two decades. Whereas there was a ten-year interval between the first three editions, the gap between the last three has narrowed to four years. Over the whole period the number of scientific terms defined has risen from 11,500 to 14,000. This latest edition contains about 500 more terms than its predecessor. As the editor points out in the preface, it would have been possible to include these additions in the form of supplements, but through the generosity of the publishers the work is completely reset for each new edition, in order to retain the alphabetical arrangement unbroken and to allow of revision, amplification and compression of the text.

The aim of the dictionary is to give the pronunciation, derivation and definition of terms in biology, botany, zoology, anatomy, cytology, genetics, embryology and physiology. It also includes a list of abbreviations occurring in biological literature and a list of equivalent units of measurement. It covers the ground with remarkable thoroughness, omissions are hard to find, and the claim to up-to-dateness seems fully justified. The work is addressed primarily to students, but also to "others interested in the Biological Sciences"—a statement which suggests that it will be of use to the layman. It succeeds admirably in its first aim, but its success in the latter seems more doubtful for, while it is true that the definitions of the less specialized scientific terms are simple and adequate, those of the more esoteric terms are often less enlightening. This is because the defining terms are as technical as the term to be defined, and in searching for the meanings of these the reader is apt to lose himself in a maze of words. The terseness of the definitions will in most cases satisfy the specialist, already familiar with the terminology employed, and obviously conciseness was dictated both by the size of the book and by the desire to avoid wasteful duplication. But I wonder whether in a work of this kind, designed as a handy reference tool, it is essential to include the other elements of traditional lexicography—pronunciation and derivation. The latter sometimes takes up more space than the definition, and the former is of doubtful use, since, as the editor rightly points out, there can never be universal agreement on this subject. I feel that the space taken up by these would have been better employed in giving longer definitions. Space could also be saved for the same purpose by grouping adjectives together at the end of the definition of the parent noun, instead of giving each a separate entry and repeating the definition every time.

But these are minor criticisms of a work which on the whole well fulfils its declared intentions, and is a reference tool of great value to anyone whose work or interests lie in the field of the biological sciences.

N. D. Blamire

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Lipid Metabolism: Introduction

The term "lipid" is useful in biochemistry, since it enables us to group together several chemically unrelated substances which are closely associated with each other in biological systems. Thus, under "lipid metabolism" we may include the metabolism of fatty acids and their esters, phospholipids, sterols and certain hydrocarbons. It will be apparent from reading this copy of the *British Medical Bulletin* that every endeavour is being made in the study of these problems to define with more and more precision the particular molecular species involved, since different, though closely similar, molecules may have markedly different biological properties and significance. In no field is this relationship between molecular structure and biological action more apparent than in the case of the sterols. It may be equally important, however, in considering fatty acids or phosphatides. Fortunately, recent advances in the techniques available for the separation, isolation, labelling, identification and quantitative analysis of the various lipids makes this more feasible than it was twenty years ago. While the term "lipid metabolism" may be conveniently retained for general use, every effort should be made, in discussing particular aspects of this subject, to define the molecules concerned as accurately as possible, using the accepted chemical terminology when this is appropriate.

Most of the main facts of lipid metabolism have been known for several decades. In some cases, however, conclusive proof was lacking and detailed information was, of necessity, based largely on conjecture. The advent of chromatographic methods of separation and isotopic labelling, together with other technological advances, has revolutionized this situation. Not only have new chemical techniques enabled us to advance our knowledge of lipid metabolism, but there have also been considerable developments on the biological side. Increasing knowledge and experience have made it possible to unravel the details of complex enzymological phenomena step by step. Not only have the chemical steps and necessary co-factors in these enzymological processes been revealed, but by improved cytological methods the distribution within the cell of the numerous enzymes concerned can also be mapped. The combination of chemical and physical methods enables us to assess the contribution made by lipids as structural units in the cell. Experimental animal preparations have been developed which can be more effectively used to solve problems. There is still some room for improvement in studies on the intact animal, especially in the more precise control of genetic and environmental factors, better appreciation of species differences and greater discrimination in the choice of animal for study. The origin and recent history of animals from which material is obtained should always be clearly defined. No one would dream of using a chemical from an unlabelled bottle, that happened to be around the laboratory, as an important component of a quantitative reaction; some animal materials would appear to be no better characterized than this. In human physiology the ultimate study must be made in man. Many problems cannot be solved by the use of normal volunteers alone. Studies in patients with carefully characterized pathological situations not only help in treatment of disease, but provide an opportunity for gaining new knowledge in human physiology. The modern developments in chemical technology, and improvements in biopsy techniques in recent years, enable us to make a wider range of human studies at the cellular level.

The papers presented here do not cover all aspects of lipid metabolism, but they touch on most of the major issues that are in the forefront of advancing knowledge at the present time. More than fifty years ago, Raper suggested that fatty acids were formed in the body ". . . by the condensation of some highly reactive substance containing two carbon atoms." In the first paper, Hele describes in considerable detail the biosynthesis of fatty acids from acetate as it can now be demonstrated in several different animal preparations. The process is broadly β-oxidation in reverse; an interesting possibility is that differentiation between anabolic and catabolic reactions may, in part, depend upon the distribution of the enzymes or co-factors between the various intracellular organelles. These studies are concerned with the biosynthesis of saturated and monoethenoid fatty acids. Lovelock & James claim that polyethenoid fatty acids, such as linoleic acid, can be synthesized by human red cells. This observation, if firmly established, raises some important issues. The formation of cholesterol in the human body was clearly indicated by the work of Gardner some thirty years ago. The detailed analysis of the pathways by which acetate is built up through mevalonic acid, squalene, and lanosterol to cholesterol, described by Cornforth & Popják, had to wait upon developments in radiochemistry. These elegant studies in biosynthesis show what can happen; for many purposes it is also important to know what does happen in the body under particular circumstances. Many factors may be concerned in the control of biosynthetic reactions—one important group is discussed by Folley & McNaught. Folley and his colleagues have developed preparations of the mammary gland for the study of biosynthetic processes. This tissue is particularly suitable for this purpose and sensitive to hormonal influences. The value of this method in sorting out the effects of hormones on lipogenesis is apparent.

The first step in the transport of exogenous lipid is absorption from the intestine. The essential intraluminar factors in fat absorption were demonstrated by Claude Bernard more than a century ago. Nearly fifty years later Pflüger introduced the concept of complete hydrolysis as an obligatory step in glyceride absorption. This view dominated work on fat absorption for forty years, but it has now been established that glycerides need not be broken down to fatty acids and glycerol before absorption can occur. This fundamental change of concept raises many further problems which are discussed elsewhere. The absorption of water-soluble substances can be studied in isolated intestinal preparations, but this has not been found to be suitable for the study of lipid absorption. The application of new methods

of chyle collection, introduction of isotopic labelling techniques, and the detailed study of the behaviour of different types of lipid molecule, have helped to advance knowledge in this field. Evidence based on studies in human subjects with defective fat absorption was cited by Verzár twenty years ago in support of various concepts, such as the importance of the adrenal cortex and phosphorylation in the fat absorption mechanism. Advances in our knowledge of the aetiology of certain enteropathies have provided the opportunity for the re-assessment of the factors concerned in human fat absorption. Another aspect of lipid absorption is considered by Glover & Morton. The absorption of sterols requires a more elaborate mechanism than that needed for paraffins. We found that cholesterol, in contrast to paraffin, was well absorbed when fed in tributyrin. Molecular structure is of great significance in sterol absorption. A link with lipoprotein formation is suggested; this may also be an important step in glyceride absorption. Lipoproteins are undoubtedly involved in the transport of lipids in the blood stream, and their circulation is discussed by French, Morris & Robinson.

The same authors also describe the handling of particulate fat in the blood stream. The existence of chylomicrons has been known for eighty years. They have been shown to consist mainly of glycerides with a lipoprotein surface film containing phosphatidylcholine, which is necessary for the stability of the fat particle in the plasma. The mechanism of their removal from the blood stream remained a mystery, however, until the accidental observation by Hahn in 1943 that heparin administration caused clearing of hyperlipaemic plasma. This effect has been extensively studied and it is now known that the clearing is brought about by a lipolytic enzyme. The fatty acid liberated is taken up by fatty acid acceptors, the most important of which is albumin. The significance of this phenomenon in lipid metabolism is discussed by French, Morris & Robinson, who conclude that, as in the case of transport of glycerides in the intestine, complete hydrolysis is not an obligatory step in the removal of glycerides from the circulation and more than one transport mechanism may exist. Apart from these problems of handling alimentary hyperlipaemia, other factors may also affect the lipid content of the blood in the post-absorptive state. Amongst these must be included hormonal factors, which are discussed on the basis of evidence from human subjects by Boyd & Oliver.

Lipids are present in all cell membranes and they also form a major component of such important organelles as mitochondria, microsomes and Golgi bodies. Only one paper in this series deals directly with this aspect of lipid metabolism. Finean & Robertson describe studies on the structure of nerve myelin in which a combination of methods has been used, including chemical analysis, polarized light microscopy, *x*-ray diffraction and electron microscopy. The results obtained by these various methods agree reasonably well. The evidence is put forward in support of the proposal that the myelin sheath is directly derived from the Schwann cell membrane and consists of layers of mesaxon wrapped spirally round the axon. If this is so, the myelin sheath is, indeed, an interesting model system!

One of the major problems of our time is the aetiology of myocardial infarction, which appears to have increased in incidence in recent years in an alarming and inexplicable way. For over twenty-five years it has been generally thought that important links exist between dietary lipids, blood lipids, atheroma and myocardial infarction. As Bronte-Stewart points out in his paper, the evidence upon which this aetiological chain is based is by no means firmly established. The effects of dietary fats upon blood cholesterol seem to be relatively well established by recent work, although the mechanism by which the changes are produced remains obscure. Agreement has not been reached on the precise aetiological relationship between blood lipids and atheroma, although few would deny its existence. The weakest link, however, is that between atheroma and myocardial infarction. Atheroma has been induced experimentally in innumerable animals, but myocardial infarction is an uncommon occurrence. It is, perhaps, as well that other aspects of this problem are receiving attention. Thus, Poole describes experimental studies on the effects of lipids on blood clotting. Again, the importance of precise definition of the lipid molecules involved is apparent. He wisely points out that blood coagulation and thrombosis are not synonymous terms.

It has been claimed recently that atheroma and myocardial infarction might be due to deficiency of essential fatty acids. The essential fatty acids are usually regarded as being linoleic, arachidonic and, possibly, linolenic acids, which will relieve the essential fatty acid deficiency syndrome in the rat.

Sinclair uses a rather wider definition. It has been shown that fats with poor essential fatty acid content by the classical definition effectively lower blood cholesterol. James & Lovelock describe analytical studies in human subjects which do not substantiate the view that there is any demonstrable deficiency of linoleic or arachidonic acid in patients with myocardial infarction. It may be pointed out, however, that there was also no significant difference in the blood cholesterol levels between these patients and the control group. They failed to find any difference in the linoleic and arachidonic acid levels between normal babies and those suffering from idiopathic hypercalcaemia which had also been attributed to essential fatty acid deficiency. The linoleic acid content of National Dried Milk, which had been cited as a causative factor, was found to be unchanged by the process used. The claim that oxidizing improving agents destroy the poly-unsaturated acids in flour has also been shown to be unfounded. James & Lovelock's claim that linoleic acid can be synthesized by human red cells does not, of course, preclude the possibility that they may still be necessary constituents in the diet. The rate of formation may not be adequate for the needs of the body. The general use of the term "essential" for certain dietary constituents may have outlived its usefulness, since needs vary from species to species and from time to time in the same animal. In spite of the failure of recent evidence to support essential fatty acid deficiency as an aetiological factor in a number of diseases, it would be unwise to sweep this problem aside too readily. The existence of an essential fatty acid deficiency syndrome in young rats is well established, and Hansen has presented evidence that effects can be brought about in babies by a diet grossly deficient in essential fatty acids. The detailed study of skin changes in essential fatty acid deficiency, as described by Sinclair, merits further attention.

As with many fields of human endeavour, the study of current achievement leads to a greater appreciation of the paucity of available knowledge and the vastness of the territories of ignorance that await an explorer. To the biologist and to the chemist, lipid metabolism still offers a promising field for adventure and discovery.

A. C. Frazer

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BIOSYNTHESIS OF FATTY ACIDS

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1 Acetate as the precursor molecule in fatty acid synthesis
2 Enzymes and co-factors concerned in fatty acid oxidation
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The β-oxidation theory of Knoop (1905) (see also Breusch, 1948) postulated that even-numbered fatty acids were broken down by the removal of successive two-carbon units from the carboxyl end of the molecule, according to the general reaction (1). The possibility has always existed that fatty acid

$$CH_3(CH_2)_{2n}COOH \longrightarrow CH_3(CH_2)_{2n-2}COOH + CH_3COOH \quad (1)$$

chains could be built up by a reversal of this process, through the successive condensation of two-carbon units (Raper, 1907; Smith & Dastur, 1938; Folley, 1949; Ochoa & Lynen, 1953). Of recent years research upon the oxidation of fatty acids has outstripped research upon their synthesis, and now a wealth of knowledge is available concerning the reactions involved in β-oxidation. This has proved of great use in the study of fatty acid synthesis, and for this reason an outline of current knowledge concerning fatty acid oxidation will be given later in this paper.

1. Acetate as the Precursor Molecule in Fatty Acid Synthesis

Rittenberg & Bloch (1945) showed, with the use of tracer techniques, that acetate was the precursor two-carbon unit involved in fatty acid synthesis. They obtained evidence that fatty acids were built up by the "head-to-tail" condensation of acetate units (see also Bloch, 1947, 1948). Further evidence that the condensation of acetate units was a successive one, and/or that it occurred from the carboxyl end of the molecule, was obtained by a number of investigators who studied the elongation of fatty acid chains. Stetten & Schoenheimer (1940) studied the conversion of deuterium-labelled palmitate to stearate, and Klem (1943) and Stevens & Chaikoff (1951) that of laurate and myristate to C_{16} and C_{18} fatty acids. Zabin (1951) and Anker (1952) showed respectively that the conversion of palmitate to stearate and of myristate to palmitate involved the condensation of C_2 units to the carboxyl end of the fatty acid molecule. For short-chain fatty acids Stadtman, Stadtman & Barker (1949) demonstrated that the conversion of butyrate to caproate by *Clostridium kluyverii* took place by a similar mechanism.

The mammary gland is unique in that it synthesizes both long- and short-chain fatty acids (C_4–C_{16}) which are secreted in the milk (Folley, 1952; Popják, 1952a; Folley, 1956). The precursor was shown to be acetate both in vitro with slices of lactating mammary gland (Folley & French, 1950; Balmain, Folley & Glascock, 1952, 1954; Popják & Tietz, 1954) and in vivo, in the pregnant or lactating rabbit (Popják & Beeckmans, 1950; Popják, Folley & French, 1949; Popják, Hunter & French, 1953), the lactating cow (Kleiber, Smith, Black, Brown & Tolbert, 1952) and the lactating goat (Popják, French & Folley, 1951). The stepwise synthesis of short-chain fatty acids from [*carboxy*-^{14}C]acetate was demonstrated in the lactating goat by Popják, French, Hunter & Martin (1951) (see Popják, 1952b, 1955).

All these investigations point to the conclusion that fatty acid chains are built up through the general reaction (2), which

$$CH_3(CH_2)_{2n}COOH + CH_3COOH \longrightarrow CH_3(CH_2)_{2n+2}COOH \quad (2)$$

would be consistent with the reversal of β-oxidation. To probe this resemblance further the study of fatty acid synthesis in cell-free or water-soluble systems is necessary.

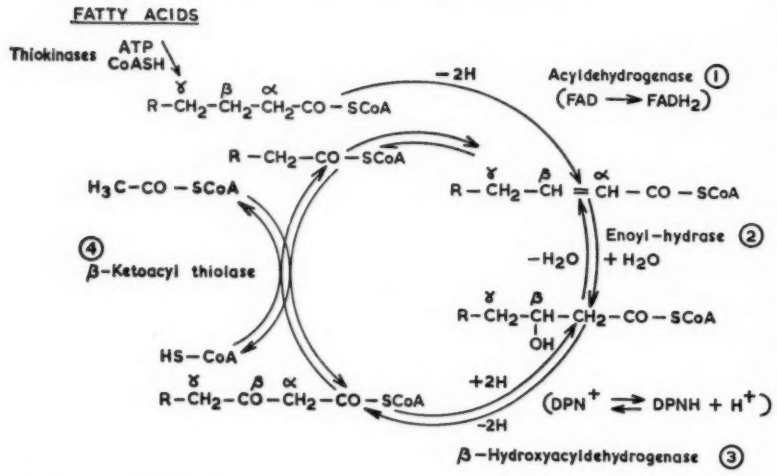
2. Enzymes and Co-Factors Concerned in Fatty Acid Oxidation[1]

The enzymes of fatty acid oxidation are concentrated in the mitochondrion (Lehninger & Kennedy, 1949) and can be extracted in water-soluble form (Drysdale & Lardy, 1953). The discovery of coenzyme A and its role in acetate metabolism (Lipmann, 1950, 1954), followed by the identification of acetyl-CoA as the actual metabolic form of acetate (Lynen, Reichert & Rueff, 1951), led to the rapid characterization and purification of the enzymes concerned in β-oxidation (Ochoa & Lynen, 1953; Green, 1954). It is now known that all even-numbered fatty acids are oxidized as their acyl-CoA derivatives (requiring initial activation at the carboxyl group with ATP) via a four-step spiral sequence, involving four enzymes, a concept made familiar by Lynen (1954) (see fig. 1).

The first oxidation step is catalysed by a group of flavoprotein enzymes (Hauge, Crane & Beinert, 1956; Crane, Mii, Hauge, Green & Beinert, 1956; Crane & Beinert, 1956), and is reversible only with dyes of low oxidation-reduction potential. The other three steps are fully and freely reversible, under conditions consistent with the equilibria of the reactions involved. It would be expected that fatty acid synthesis could be brought about by a reversal of the "spiral", an idea which was tested by Stansley & Beinert (1953), using highly purified enzymes obtained from beef-liver mitochondria. They met with relative failure; starting with acetyl-CoA, only small quantities of butyrate were formed, and no higher fatty acids. This failure was attributed to the fact that the β-ketoacyl thiolase (the enzyme catalysing step 4 in fig. 1) used was incapable of condensing higher acyl-CoA's with acetyl-CoA (Green, 1956).

[1] The following abbreviations will be used in this paper: coenzyme A, CoA; adenosine triphosphate, ATP; diphospho- and triphosphopyridine nucleotides, DPN and TPN; their reduced form, DPNH and TPNH; glutathione, GSH; cysteine, CSH.

FIG. 1. THE FATTY ACID CYCLE



R represents $CH_3(CH_2)_{2n}$.

Circled numbers 1, 2, 3, 4, refer to the four main steps of the cycle catalysed by the four principal enzymes.

3. Fatty Acid Synthesis in Water-Soluble Systems

a. General Considerations

The study of fatty acid synthesis has moved rapidly from the tissue slice (see Bloch, 1952) to the water-soluble, or partially water-soluble system. Only a relatively small amount of work has been done using tissue homogenates (Bucher, 1953; Popják & Tietz, 1954; Terner, 1954).

A variety of tissues have yielded systems synthesizing fatty acids, each showing its own characteristic properties (Table I). Nevertheless, certain common characteristics have emerged. All systems have a requirement for the co-factors associated with fatty acid oxidation: ATP, CoA, DPN, and Mg^{++}. All systems require the addition of an oxidizable substrate if fatty acid synthesis is to proceed, the exceptions being those systems in which stoicheiometric quantities of reduced pyridine nucleotides are added. All investigations have revealed that mitochondria alone are incapable of carrying out fatty acid synthesis, although containing the full complement of enzymes required for fatty acid oxidation. Finally, it now appears that fatty acid synthesis differs from oxidation in at least one important essential, in requiring TPNH for the reduction of unsaturated to saturated fatty acid. The enzyme system that performs this reaction is extra-mitochondrial, and is probably located in the microsome.

b. Pigeon-Liver Systems

Brady & Gurin (1952) studied the incorporation of [*carboxy*-^{14}C]acetate into long-chain fatty acids, in a system composed of a "high-speed supernatant"[2] reinforced with a water-soluble extract of acetone-dried mitochondria. Mg^{++} markedly stimulated incorporation, α-oxoglutarate being without effect. A requirement for DPN, ATP and CoA was demonstrated after treatment of the liver extracts with charcoal (Van Baalen & Gurin, 1953), but no clear TPN requirement was established. Brady, Mamoon & Stadtman (1956) showed that the fatty acid synthesizing activity of their pigeon-liver preparation was concentrated entirely in the high-speed supernatant fraction. The mitochondrial fraction was incapable of synthesis, although it oxidized [^{14}C]palmityl-CoA. Incorporation of [*carboxy*-^{14}C]acetate into long-chain fatty acids by the high-speed supernatants was stimulated by citrate, or by *iso*citrate, which was shown to generate TPNH. α-Oxoglutarate or glucose 6-phosphate were separately unable to stimulate incorporation, but when added together were as satisfactory as *iso*citrate. The reported failure of acetyl-CoA to act as precursor (Brady & Gurin, 1952) was shown to be due to the inhibitory effect upon acetate incorporation of free CoA, liberated by deacylase action upon the acetyl-CoA.

A highly purified enzyme system has been obtained from pigeon liver (Gibson, Jacob, Porter, Tietz & Wakil, 1957; Wakil, Porter & Gibson, 1957) or chicken liver (Tietz, 1957), capable of synthesizing long-chain fatty acids, predominantly palmitate, from [*carboxy*-^{14}C]acetate without any appreciable formation of short-chain intermediates, except for β-hydroxy-butyrate and crotonate. Short-chain saturated fatty acids appeared when [*carboxy*-^{14}C]acetyl-CoA was used (Porter & Tietz, 1957). A net synthesis of long-chain fatty acid was obtained, in which the isotope distribution suggested synthesis via the successive condensation of acetate units. An absolute requirement for ATP, CoA, DPNH, Mn^{++}, GSH and *iso*citrate was demonstrated, whereas in the absence of TPN 50% of the activity still persisted. High concentrations of CoA proved to be inhibitory (Porter, Wakil, Tietz, Jacob & Gibson, 1957). Glucose 6-phosphate, α-oxoglutarate and HCO_3, an *iso*citrate-generating system, would replace *iso*citrate. It appears that *iso*citrate exerts a stimulating effect on the system over and above that of its role in TPNH generation.

Various enzymes have been detected in the fractions, the most interesting being a β-ketoacyl thiolase capable of condensing long-chain fatty acyl-CoA's with acetyl-CoA (compare the experiment of Stansley & Beinert, 1953), and a "reductase" catalysing the reduction of crotonyl-CoA with TPNH. Enzymes of β-oxidation have also been detected.

c. Mammary Gland Systems

Popják & Tietz (1955) obtained a high-speed supernatant fraction from lactating rat mammary gland capable of incorporating [*carboxy*-^{14}C]acetate into predominantly short-chain fatty acids (C_4–C_8). In contrast, the homogenates, from which the supernatants were prepared, incorporated [*carboxy*-^{14}C]acetate into predominantly long-chain fatty acids (C_{10}–C_{18}) (Popják & Tietz, 1954). Addition of mitochondria to the supernatant fraction profoundly depressed incorporation, whilst the addition of the microsome fraction had a variable, small, depressing effect. Oxaloacetate and α-oxoglutarate stimulated incorporation, and the stimulating effect of the

[2] "High-speed supernatant" refers to material obtained by centrifuging a tissue homogenate at 100,000 *g* for at least 30 minutes.

TABLE I. SOME CHARACTERISTICS OF SYSTEMS SYNTHESIZING FATTY ACIDS FROM [^{14}C]ACETATE

Tissues and nature of systems investigated	Pigeon liver		Mammary gland		Rat liver	
					Unpurified water-soluble system	
	Unpurified water-soluble system	Purified system	Unpurified water-soluble system	Partially purified system	Langdon (1957)	Dituri, Shaw, Warms & Gurin (1957)
Tissue fractions employed	Mitochondrial extracts + high-speed supernatant*, or high-speed supernatant† alone	Purified protein fractions from high-speed supernatant	High-speed supernatant; system inhibited by mitochondria; microsomes had little effect	Partially purified from high-speed supernatant	High-speed supernatant; system slightly stimulated by mitochondria; microsomes had no effect	Mitochondrial extracts or mitochondria + high-speed supernatant or high-speed supernatant alone
Fatty acids formed	Long-chain (palmitate carrier used for isolation)	Long-chain; net synthesis of predominantly palmitate	75% Short-chain (C_4–C_{10})	90% Short-chain (C_4–C_{10})	Predominantly long-chain; $>C_8$	Long-chain (palmitate carrier used for isolation)
Co-factors required	ATP, CoA, DPN, Mg^{++}	DPN, ATP, CoA, GSH, Mn^{++} (TPN)	CoA, ATP, DPN	CoA, ATP, DPNH, CSH, Mg^{++}	DPN, TPN, CoA	DPN, CoA, ATP, Cu^{++}, Zn^{++}
		Insulin‡, lipoic acid		TPN requirement impossible to demonstrate		
		Inhibited by high concentrations of CoA		Requires high concentrations of CoA		
Oxidizable substrate or TPNH-generating system promoting synthesis	Citrate*, *iso*citrate†, glucose 6-phosphate + α-oxoglutarate together	*Iso*citrate, glucose 1-phosphate, glucose 6-phosphate + α-oxoglutarate together	α-Oxoglutarate, oxaloacetate (malonate stimulates powerfully)	—	*Iso*citrate, glucose 1-phosphate	Citrate, glucose 6-phosphate + α-oxoglutarate together
Oxidizable substrate or TPNH-generating system having no effect	Glucose 6-phosphate or α-oxoglutarate separately	Glucose 6-phosphate or α-oxoglutarate separately	Citrate	—	—	Glucose 6-phosphate
Oxygen requirement	Aerobic (anaerobiosis only slightly inhibitory)	Anaerobic	Aerobic or anaerobic	Aerobic (no precautions taken to ensure anaerobiosis)	Anaerobic	Aerobic, 95% O_2
Intermediates formed, or employed	—	β-Hydroxybutyrate and crotonate detected; short-chain saturated fatty acids detected with acetyl-CoA as substrate	—	Acyl-CoA derivatives similar to those occurring during fatty acid oxidation	Synthetic CoA derivatives employed	Labelled β-hydroxybutyrate detected

* **Brady & Gurin (1952)**

† **Brady, Mamoon & Stadtman (1956)**

‡ **Crude preparations**

latter was greatly enhanced by the presence of malonate. Citrate was without effect. An absolute requirement for ATP was observed and, after treatment with Dowex-1 ion-exchange resin, a requirement for DPN and CoA emerged (Tietz & Popják, 1955).

Hele, Popják & Lauryssens (1957) obtained a fatty acid synthesizing system from the high-speed supernatant from lactating rabbit mammary gland, which they partially purified by ammonium sulphate fractionation. Predominantly short-chain fatty acids were synthesized. This system had a requirement for ATP, CoA, Mg^{++}, cysteine and DPNH. Repeated experimentation failed to show a requirement for TPN. The course of [*carboxy*-^{14}C]acetate incorporation into fatty acids followed that of DPNH utilization as measured in the spectrophotometer. Intermediates accumulated, which were studied by the paper chromatography of their hydroxamates (Hele & Popják, 1956) (fig. 2). It was found that β-keto acids, β-hydroxy acids and unsaturated fatty acids were formed during the course of the reaction, and preceded the formation of the appropriate saturated fatty acid. Certain of these intermediates were later shown by de Waard & Popják (1958) to be CoA derivatives. This is the only direct experimental evidence which shows that the fatty acid substrates involved in fatty acid synthesis go through a series of steps which are the exact reversal of those occurring during fatty acid oxidation.

d. Rat-Liver Systems

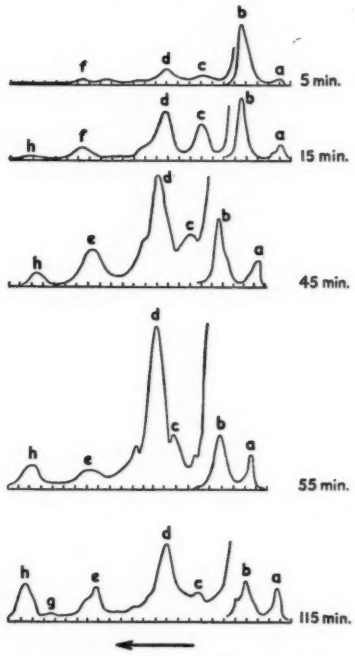
Langdon (1955, 1957) was the first to make the important observation that the reduction of unsaturated fatty acid to saturated fatty acid required TPNH as an electron donor. The high-speed supernatant employed by him was apparently free of microsomes. Addition of microsomes was without effect upon the total incorporation of [*carboxy*-^{14}C]acetate into long-chain fatty acids, whilst the addition of mitochondria had a slight stimulating effect. A requirement for DPN, TPN, and CoA was demonstrated, although one for ATP was never detected. TPNH was either generated via *iso*citrate, or added in stoicheiometric quantities in those experiments in which the direct spectrophotometric reduction of crotonyl-CoA or hex-2-enoyl-CoA was demonstrated. DPNH was completely without effect in this reaction. The reverse reaction, butyryl-CoA + TPN ⟶ crotonyl-CoA + TPNH did not occur. The following oxidative enzymes were shown to be present in the rat-liver high-speed supernatant: butyryl dehydrogenase, crotonase, and β-hydroxyacyldehydrogenase.

Dituri, Shaw, Warms & Gurin (1957) have prepared a system from rat liver which resembles in all its essentials the pigeon-liver system of Brady & Gurin (1952). They observed that the soluble lipogenic fraction of the mitochondria was extracted into the high-speed supernatant fraction when electrolytes were present in the homogenization medium. Citrate was required if the synthesis was to progress beyond the stage of β-hydroxybutyrate. Washed mitochondrial particles could synthesize long-chain fatty acids from [2-^{14}C]pyruvate, provided that citrate and butyryl-CoA were added (Shaw, Dituri & Gurin, 1957).

e. Mixed Systems

Evidence has been obtained that would point to the microsome as being an important site of reductive syntheses. Lachance, Popják & de Waard (1958) studied the synthesis of butyrate from crotonate, in a system consisting of rat-liver

FIG. 2. RADIOCHROMATOGRAM TRACING SHOWING THE FORMATION OF ^{14}C-LABELLED INTERMEDIATES (AS HYDROXAMATES) DURING FATTY ACID SYNTHESIS (see Hele & Popják, 1956)



The time in minutes refers to the time from the start of the experiment at which the samples were drawn.

The horizontal scale indicates the length of the chromatograms in cm., and the arrow the direction of solvent flow.

The areas under the curves are proportional to the ^{14}C content of the substances in the chromatographic spots.

a: β-hydroxybutyrate	e: β-hydroxyhexanoate
b: acetate	f: β-hydroxyoctanoate
c: crotonate	g: hexanoate
d: butyrate	h: octanoate and higher acids

The vertical scale for peaks (a) and (b) is $\frac{1}{20}$ of that for peaks (c) to (h). Peak (a) at 5 min. represents about 2 μmoles of acetate. Peak (d) at 55 min. represents about 0.1 μmole of butyrate.

microsomes—isolated according to Bucher & McGarrahan (1956)—added to a crotonyl-CoA synthesizing system obtained from lactating rabbit mammary gland. Only very small amounts of butyrate were formed in the absence of either TPNH (generated via glucose 6-phosphate) or microsomes. [*Carboxy*-^{14}C]acetate, CoA and ATP, or [^{14}C]crotonyl-CoA served as precursors. Owing to the DPNH oxidase in the microsomes repeated additions of DPNH had to be made, and even this was insufficient to prevent the total synthesis of unsaturated + saturated fatty acid from being depressed. TPNase in the microsomes was effectively countered with nicotinamide. Isolation of the products of the enzymic reactions and a direct spectrophotometric test indicated that the microsome fraction catalysed the conversion of crotonyl-CoA to butyryl-CoA at the expense of TPNH. The mammary

gland preparation catalysed the oxidation of butyryl-CoA to acetyl-CoA.

Seubert, Greull & Lynen (1957) isolated a particulate fraction from pig-liver mitochondria by treatment with cholate. This preparation contained none of the enzymes of fatty acid oxidation, but would catalyse the conversion of oct-2-enoyl-CoA to octanoyl-CoA with TPNH. This reaction was demonstrated in the spectrophotometer. A combination of this particulate preparation with the highly purified enzymes of β-oxidation catalysing reactions 2, 3, 4 (fig. 1) would bring about the conversion of hex-2-enoyl-CoA + [*carboxy*-^{14}C]acetyl-CoA to fatty acids of chain-length up to C_{16}, in the presence of TPNH and the other appropriate co-factors.

4. Fate of Fatty Acids after Synthesis

The fatty acids synthesized in vitro appear to be either free fatty acids, or in combination with CoA as their acyl-CoA derivatives (e.g. Van Baalen & Gurin, 1953; Porter & Tietz, 1957). In these systems it is probably the chain-length specificity of the deacylating enzymes that determines when the fatty acyl-CoA's leave the cycle of synthesis. The deacylating reaction (3) can be represented as the hydrolysis of

$$CH_3(CH_2)_{2n}CO{-}S{-}CoA + H_2O \longrightarrow CH_3(CH_2)_{2n}COOH + HSCoA \quad (3)$$

the thio-ester bond of the acyl-CoA. In vivo the fatty acids probably leave the cycle to become glycerides, perhaps first condensing with α-glycerophosphate (derived from carbohydrate (see Popják, 1955)) to give phosphatidic acids (Kornberg & Pricer, 1953). This reaction (4) has its optimum

$$\begin{array}{l} H_2COH \\ | \\ HCOH \\ | \\ H_2COPO_3H_2 \end{array} + 2CH_3(CH_2)_{2n}CO{-}S{-}CoA \longrightarrow \begin{array}{l} H_2COOC(CH_2)_{2n}CH_3 \\ | \\ HCOOC(CH_2)_{2n}CH_3 \\ | \\ H_2COPO_3H_2 \end{array} + 2HSCoA \quad (4)$$

with fatty acids containing 16 or 18 carbon atoms; this perhaps explains the preponderance of these fatty acids in animal tissues. Diglycerides can arise from these phosphatidic acids, and give rise to triglycerides after combining with a further molecule of fatty acyl-CoA. The diglycerides also serve as precursors of phosphatides. A summary of these interconversions is given by Kennedy (1957).

5. Summary and Conclusions

The separation, or partial separation, of the pathway of fatty acid synthesis from the pathway of fatty acid oxidation probably reflects the "reducing environment" or "oxidizing environment" required for each process, a situation fully discussed by Langdon (1957), and related to the intramitochondrial and extra-mitochondrial concentration of reduced pyridine nucleotides. TPNH may function as the specific electron donor of many reductive syntheses (Krebs, 1954); indeed TPN exists in tissues predominantly in the reduced form (Glock & McLean, 1955). The intriguing possibility has now arisen that the microsome plays an important part in these reductive syntheses (Lachance *et al.* 1958).

Whether or not divergences between synthesis and oxidation occur at other points on the fatty acid cycle cannot at the moment be decided, since the real source of the enzymes present in the supernatant fractions which catalyse fatty acid synthesis is not known. It seems fairly clear that the fatty acid substrates themselves undergo a series of changes which are an exact reversal of events taking place during oxidation, and that if an extra-mitochondrial group of enzymes exist that catalyse steps 2, 3, 4 (fig. 1) in reverse, then they must differ from their mitochondrial counterparts in a rather subtle manner, as might the β-ketoacyl thiolase cited by Green (1956). It is of interest to recall here that the purified enzymes employed by Stansley & Beinert (1953) were specifically isolated from mitochondria; whilst those used by Seubert *et al.* (1957) were probably prepared from whole sheep liver (Lynen, Wessely, Wieland & Rueff, 1952). The extra-mitochondrial concentration of DPNH (which might be maintained via the action of transhydrogenase (Kaplan, Colowick & Neufeld, 1953) would also favour the first reduction step, that of a β-keto acid to the β-hydroxy acid.

The system in the mammary gland (Popják & Tietz, 1955; Hele *et al.* 1957) which appears to synthesize only short-chain fatty acids remains something of an anomaly. Its characteristics differ so markedly from those of the liver systems (see Table I) that it is tempting to regard it as a system specific to the mammary gland, responsible for the synthesis of the short-chain fatty acids of the milk, and utilizing DPNH for both reduction steps. The long-chain fatty acid synthesizing system may prove upon investigation to be TPNH-dependent. The two systems may function quite separately: a neat way of overcoming the necessity for tapping the long-chain fatty acid synthesizing system of its short-chain fatty acid precursors.

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HORMONAL EFFECTS IN VITRO ON LIPOGENESIS IN MAMMARY TISSUE

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The scope of this article has necessarily been confined within rather narrow limits. In discussing the effect of hormones on lipogenesis we have restricted ourselves mainly to a discussion of effects obtained in vitro with mammary gland tissue.

This tissue possesses two properties which make it particularly suitable for studying hormonal effects on lipogenesis in vitro. First, during its functional phase, just as much as during its phase of growth, it is a target organ for a number of hormones—during the secretory phase in particular, perhaps the most comprehensive target organ of any in the body. Second, it is a site of very active synthesis of protein, carbohydrate and, what is of particular concern to us here, of fat. It is not surprising, therefore, that study of mammary tissue has proved fruitful not only for those whose interest lies primarily in the biochemistry of milk secretion, but also for those biochemists interested in the study of synthetic processes. Of these, lipogenesis has received much attention in recent years, and our understanding of the biochemical mechanism of this process is relatively advanced.

1. Fatty Acid Synthesis in Mammary Gland Slices

a. Respiration Studies

In our laboratory it was shown about ten years ago that lactating mammary gland slices from various species, when incubated with suitable substrates, exhibit a respiratory quotient (R.Q.) appreciably greater than unity (Folley & French, 1949, 1950). Thus mammary gland slices from non-ruminants (mouse, rat, guinea-pig and rabbit) were found to be capable of utilizing glucose with R.Q. values ranging from about 1.17 in the case of the guinea-pig, to about 1.90 in the case of the mouse, whereas mammary gland slices from lactating ruminants (sheep, goat and cow) gave R.Q. values less than unity (see Table I). On the other hand, in the presence of acetate alone the R.Q. exhibited by mammary tissue from non-ruminants, as exemplified by the rat, was now less than unity, and the oxygen consumption ($-Q_{O_2}$) was little if at all greater than the endogenous values found in the absence of substrates, but values greater than unity were given by ruminant slices. It was also shown that, in the presence of both glucose and acetate, non-ruminant tissue utilizes some acetate, while the acetate uptake of ruminant tissue is markedly enhanced. These high R.Q. values exhibited by mammary gland slices were interpreted as indicating that this tissue was capable of effecting net synthesis of fat from small molecules—glucose and perhaps acetate in the case of non-ruminant mammary tissue, and acetate in the case of ruminant mammary gland.

b. Isotope Studies

The above interpretation was substantiated and various aspects further elucidated by later experiments in which mammary gland slices were incubated with isotopically labelled substrates and the isotope content of the mixed fatty acids, obtained from the slices at the end of the experiment, determined. In these experiments (Balmain, Folley & Glascock, 1952a, 1954) it was shown that rat mammary gland slices incubated with glucose plus acetate actively incorporated acetate and glucose carbon into fatty acids at approximately equal rates, whereas with ruminant mammary slices, as exemplified by tissue from the lactating ewe, the incorporation of acetate carbon under similar conditions was about 30 times as fast as that of glucose carbon, the incorporation of which was relatively trivial. The fact that mammary slices were capable of effecting net fat synthesis in vitro was, therefore, placed beyond doubt. Later it was shown by Popják & Tietz (1954), and in our laboratory by Terner (1956), that cell-free homogenates of lactating mammary tissue would incorporate

TABLE I. EFFECT OF DIFFERENT SUBSTRATES AND OF INSULIN ON THE RESPIRATORY QUOTIENT (R.Q.) OF LACTATING MAMMARY GLAND SLICES FROM VARIOUS SPECIES

Animal	Substrate	Respiratory quotients	
		Without insulin	With insulin
Mouse	Glucose	1.90[b]	2.14[b]
	Glucose+acetate	1.46[b]	2.14[b]
Rat	Glucose	1.57[a]	1.80[a]
	Acetate	0.82[a]	—
	Glucose+acetate	1.53[a]	2.03[a]
Guinea-pig	Glucose	1.17[c]	—
Rabbit	Glucose	1.30[d]	—
	Acetate	0.92[d]	—
	Glucose+acetate	1.24[b]	1.67[b]
Sheep	Glucose	0.88[d]	—
	Acetate	1.09[b]	1.09
	Glucose+acetate	1.52[b]	1.50[b]
Goat	Glucose	0.86[c]	—
	Acetate	1.17[d]	—
Cow	Glucose	0.84[d]	—
	Acetate	1.12[d]	—

[a] Balmain, French & Folley (1950)
[b] Balmain & Folley (1951)
[c] Folley & French (1949)
[d] Folley & French (1950)

acetate into long-chain fatty acids. More recently Popják & Tietz (1955) succeeded in preparing, from lactating rat mammary gland, a water-soluble enzyme system capable of catalysing the formation of fatty acids of various chain-length from acetate. A partially purified enzyme system from rabbit mammary gland was then isolated and studied by Hele & Popják (1955) and Hele, Popják & Lauryssens (1957).

2. Effect of Hormones on Fatty Acid Synthesis

a. Insulin

That insulin is concerned in the synthesis of fat from carbohydrate in the whole animal has been known for a good number of years, from experiments in vivo (see, for example, Drury, 1940) in which it was shown that lipogenesis from carbohydrate was impaired in the animal rendered experimentally diabetic, and could be restored by the administration of insulin. Bloch & Kramer (1948) were the first to demonstrate, however, a stimulating effect of insulin added in vitro on lipogenesis in a mammalian tissue. They showed that, in slices of rat liver incubated in the presence of acetate and pyruvate, addition of insulin to the medium caused an increased incorporation of labelled acetate carbon into the fatty acids of the slices. Since at about this time we had just become aware of the outstanding capabilities of mammary gland slices for effecting net fatty acid synthesis in vitro, as indicated above, the work of Bloch & Kramer (1948) prompted us to investigate the effect of insulin, added in vitro, on lipogenesis in this tissue. We found (see Table I) that addition of crystalline insulin, free from glucagon, to the medium in which lactating rat mammary gland slices were incubated with glucose or glucose plus acetate as substrates, caused a marked and immediate increase in the R.Q. and in $-Q_{O_2}$ and also, when acetate was present, an increase in the acetate uptake (Balmain, French & Folley, 1950). These results were confirmed and extended in later respiratory studies on lactating rat mammary tissue (Balmain & Folley, 1951) in which it was also shown that insulin caused an increase in the uptake of glucose from the medium. In addition, insulin was shown to cause an increase in the R.Q. of mammary slices taken from the rabbit and the mouse. However, lactating mammary tissue from ruminants (sheep) was completely insensitive to the stimulating action of insulin either in the presence of acetate or of acetate plus glucose (see Table I). It was also found that rat mammary gland tissue was sensitive to insulin only in the phase of active lactation and that tissue taken at the end of pregnancy or during the early stages of weaning was completely unaffected by the presence of insulin in the medium. These unequivocal effects of insulin, added to the medium, on the metabolism of lactating rat mammary gland slices, consisting of considerable increases in R.Q., $-Q_{O_2}$, and in the uptake of glucose and of acetate if present, were interpreted by us as a manifestation of an increase in the rate of net fat synthesis from small molecules. Hills & Stadie (1952) confirmed these findings with rat mammary gland slices and showed furthermore that contact of the slices with insulin for only 20 seconds was sufficient to produce the effect in the subsequent incubation period. In later work involving the use of isotopically labelled substrates (Balmain *et al.* 1952a; Balmain, Folley & Glascock, 1954), it was found that addition of crystalline insulin to the medium, in which rat mammary gland slices were incubated in the presence of labelled acetate and glucose, caused an increase in the incorporation of carbon from both sources into the total fatty acids of the slices (see Table II). However, in the case of mammary gland slices from lactating ewes, insulin had no effect whatever on the incorporation of acetate or glucose into fatty acids of the slices. These observations on the effects of insulin on the rate of incorporation of acetate and glucose carbon into fatty acids thus substantiate the deductions drawn from the previous respiration studies.

TABLE II. EFFECT OF INSULIN ADDED IN VITRO ON LIPOGENESIS IN LACTATING MAMMARY GLAND SLICES INCUBATED WITH $C^3H_3{}^{13}COONa$ (0.02 M) + ^{14}C-GLUCOSE (0.3%) (Results from Balmain, Folley & Glascock, 1954)

Source of tissue	Addition	Isotope content of Ca salts of mixed fatty acids after 3 hours		
		^{14}C (counts/min./mg. C)	^{3}H (counts/min./mg. water of combustion)	^{13}C (atom-% excess)
Rat	None	381	95	0.054
	Insulin (1 i.u./ml.)	805	202	0.14
Sheep	None	22.5	501	0.373
	Insulin (1 i.u./ml.)	25.3	485	0.334

The interesting species differences as regards the effect of insulin on lipogenesis in vitro in mammary tissue from the rat on the one hand, and the sheep on the other, indicate that the stimulating effect of insulin on lipogenesis must be bound up in some way with the effect of insulin on the utilization of glucose by the tissue. The effect of insulin on the relationship between glucose utilization and fatty acid synthesis in rat mammary gland has recently been investigated by Abraham, Cady & Chaikoff (1957). From their studies involving the use of specifically labelled glucoses they conclude that both the Embden-Meyerhof glycolytic pathway and a direct oxidative pathway—probably the pentose cycle—provide C_2 units for the synthesis of fatty acids. Insulin increased the amount of glucose carbon converted to fatty acid carbon via both these pathways, but did not change the relative proportions in which they contributed carbon for fatty acid synthesis. It would seem therefore that the species differences found between ruminant and non-ruminant tissue as regards stimulation of lipogenesis by insulin cannot be explained in terms of these different pathways of glucose utilization.

It is of interest to digress a little at this point to consider the possibility that the stimulating effect of insulin on lipogenesis and hence on the gaseous metabolism of mammary gland slices in vitro might be used as the basis of a sensitive method for the assay of insulin in vitro. Such a method might perhaps be useful for determining the insulin content of blood and other biological fluids. Methods based on the stimulating effect of insulin on the glucose uptake of the rat hemidiaphragm in vitro have been used by many investigators for this purpose (see Randle, 1957).

If slices of lactating rat mammary gland are incubated in the Warburg apparatus in Krebs bicarbonate saline containing glucose or glucose plus acetate as substrate, the gas phase being 95% oxygen plus 5% carbon dioxide, the total pressure undergoes a steady increase from the outset of the experiment. This occurs principally by virtue of the fact that under these conditions, as stated above, the tissue exhibits an R.Q.

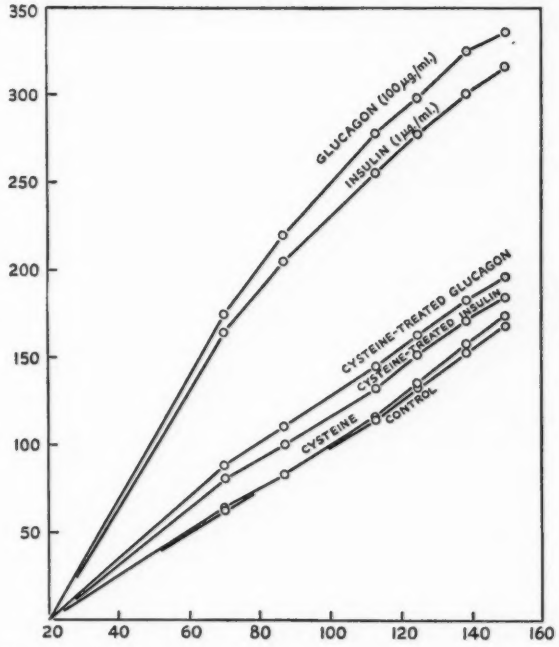
appreciably greater than unity, so that in a given time considerably more carbon dioxide is evolved than oxygen is consumed. Under these conditions the curve of over-all pressure change—the "composite respiration curve" as it is called—for mammary tissue has a positive slope. It is easy to see that, since addition of insulin to the medium causes an appreciable increase in the R.Q., the slope of the composite respiration curve, and hence the over-all gas output in a given time, is also markedly increased in the presence of insulin. We have studied the possibility of utilizing this effect of insulin as the basis of a convenient in-vitro method of insulin assay, and our results established the feasibility of such a method, at any rate as applied to solutions of purified insulin (Balmain, Cox, Folley & McNaught, 1954). It is, of course, equally possible to use the effect of insulin on the glucose uptake of the lactating rat mammary slice for this purpose since this can be determined in the same experiments, and assays based on both effects have indeed been used by us for the investigation of the insulin content of glucagon preparations as described below. The minimum amount of insulin detectable by our method is in the neighbourhood of 0.1 μg. or 2 milli-units; thus it does not appear to be as sensitive as some of the methods based on the glucose uptake of the rat diaphragm in vitro, judging by the values of the minimum detectable amounts of insulin (0.005–0.10 milli-units) listed by Randle (1957).

b. Glucagon

The recent isolation in pure and crystalline form of glucagon, the hyperglycaemic factor secreted by the α-cells of the pancreas, which is known to exert effects on carbohydrate metabolism in the whole animal in many ways opposite to those of insulin, raises the interesting question as to what, if any, are the effects of glucagon, added in vitro, on lipogenesis in mammary gland tissue. Would glucagon by itself inhibit lipogenesis in mammary tissue, would it merely antagonize the effect of insulin or would it have no effect? In order to investigate this question we have studied the effect of crystalline glucagon (lot no. 258–234B–43, Eli Lilley & Co.) added to the medium on the composite respiration curve of slices of the lactating mammary gland of the rat, and have observed (Folley and McNaught, unpublished work) that additions of upwards of 10 μg. glucagon/ml. of medium increased the slope of the composite respiration curve. These effects, therefore, resembled those of insulin in that the net gas output of the mammary gland tissue was increased. In further experiments it was found that glucagon increased the R.Q. and oxygen consumption of lactating rat mammary gland slices just as did insulin.

Experiments were then designed to throw light on the question whether the effect of glucagon on the metabolism of mammary slices might be due to insulin contamination. Use was made of the fact that, by contrast with insulin, the biological activity of which is abolished by treatment with thiol compounds, glucagon does not undergo inactivation when treated in this way. We found that our preparation of crystalline glucagon, after treatment with cysteine, no longer increased the slope of the composite respiration curve (fig. 1) or stimulated the glucose uptake of lactating rat mammary gland slices. These results suggested that the insulin-like effect might indeed be due to a small amount of insulin present in the glucagon preparation. The manufacturers originally stated that the contamination of the glucagon preparation with insulin amounted to less than 0.05 unit/mg., i.e., approxi-

FIG. 1. ABOLITION OF THE STIMULATING EFFECT OF INSULIN AND GLUCAGON PREPARATIONS ON THE COMPOSITE RESPIRATION CURVE OF LACTATING RAT MAMMARY SLICES, BY TREATMENT WITH CYSTEINE



Abscissae: time (min.)

Ordinates: over-all gas output calculated as μl. CO_2/150 mg. moist slices

mately 0.25% as determined by the mouse convulsion test. However, comparison of the magnitude of the above-mentioned effects of glucagon on the composite respiration curve, with those of insulin (see fig. 1), suggested that they were much larger than could be accounted for by the amount of insulin said to be present. To obtain a more precise value for the insulin activity of the glucagon preparation, a six-point assay was carried out by the method of Balmain, Cox, Folley & McNaught (1954) against a crystalline preparation of insulin virtually free from glucagon. In this assay, done in collaboration with Miss P. M. Clarke, the changes both in the over-all gas output and in the glucose uptake of the slices were measured to give independent estimates of the potency ratio. The results, which are given in Table III, show that,

TABLE III. ASSAY OF INSULIN ACTIVITY OF CRYSTALLINE GLUCAGON BY THE METHOD OF BALMAIN, COX, FOLLEY & McNAUGHT (1954)

Response metameter	Insulin in glucagon (%)	5% fiducial limits
Log of gas output . .	1.08	0.78–1.50
Glucose uptake . . .	1.13	0.79–1.61

on the assumption that the effects of glucagon in stimulating the glucose uptake of rat mammary gland slices and increasing the slope of the composite respiration curve are due to insulin in the preparation, then the amount present was approximately 1%, and therefore four times that stated by the manufacturers.

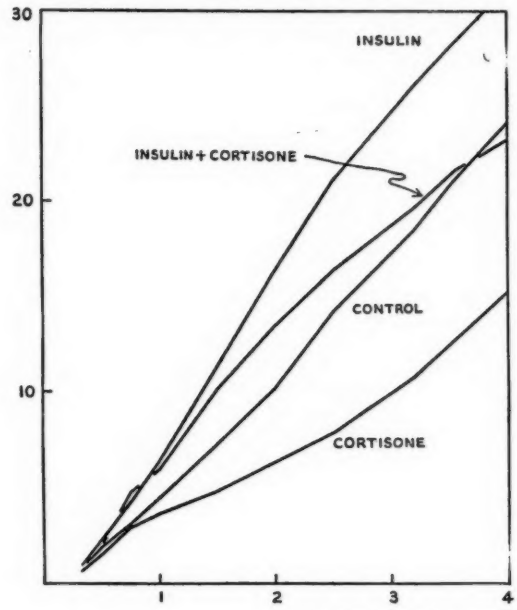
No evidence was obtained to indicate that glucagon, in the concentrations used, exerts any effects on the metabolism of lactating rat mammary slices in vitro, detectable by our techniques. Since there is ample evidence that changes in the rate of lipogenesis of this tissue are reflected in its respiratory metabolism, it seems fairly safe to conclude that glucagon, under our conditions, is without effect on this process.

c. Steroid Hormones of Adrenal Cortex

The antagonism between adrenal corticoids and insulin as regards carbohydrate metabolism in the living animal is well known. Thus, we know on the one hand that insulin is an antidiabetogenic hormone, while on the other hand certain steroids secreted by the adrenal cortex, the glucocorticoids, exert diabetogenic effects. Further light on this physiological antagonism between insulin and glucocorticoids would be shed if it were possible to study effects of these hormones on isolated enzyme systems. However, no wholly substantiated effects of insulin on lipogenesis in cell-free systems have so far been reported, although Green (1956) cites unpublished experiments of D. M. Gibson and J. W. Porter which show stimulation by insulin of fatty acid synthesis from acetate in a soluble enzyme system. As a first step towards this objective, therefore, we have carried out experiments on slices of lactating rat mammary tissue in which, as we have seen, the process of lipogenesis is readily susceptible of study by various techniques which clearly demonstrate the marked influence of insulin upon this process. In preliminary experiments (Balmain, Folley & Glascock, 1952b), in which the composite respiration curve technique was used, we found that cortisone was capable not only of depressing the slope of the composite respiration curve of lactating rat mammary gland slices, but also of partially inhibiting the stimulatory action of insulin. Conversely, in the presence of sufficient insulin the inhibitory action of cortisone could be abolished (see fig. 2).

In subsequent experiments (McNaught, Glascock, Balmain & Folley, 1955), the action of three glucocorticoids (cortisol, cortisone and corticosterone) and the mineralocorticoid (11-deoxycorticosterone) added in vitro, on the incorporation of labelled acetate or glucose carbon into the fatty acids of rat and sheep mammary gland slices, was studied. Confirming the preliminary results obtained by the composite respiration curve technique it was found that cortisone exerted a definite but relatively weak inhibitory action on lipogenesis from both acetate and glucose carbon in rat mammary gland slices. Corticosterone and 11-deoxycorticosterone, however, were much more active in this respect, while cortisol by contrast was almost totally inactive at the concentrations used, namely 50–100 μg./ml. The 17α-hydroxy group seems to suppress the inhibitory effect of these steroids on lipogenesis under these conditions. It was further found that the stimulatory effect of insulin on the incorporation of acetate and glucose carbon into fatty acids of rat mammary gland slices could be wholly or partially suppressed by suitable concentrations of corticoids, particularly corticosterone and 11-deoxycorticosterone. Inhibitory effects were also found on lipogenesis from acetate in sheep mammary gland slices, though there

FIG. 2. ANTAGONISTIC EFFECTS OF CORTISONE AND INSULIN ON THE GAS EXCHANGE OF LACTATING RAT MAMMARY GLAND SLICES



Balmain, Folley & Glascock (1952b) by permission of Nature

Abscissae: time (hr.)

Ordinates: over-all gas output calculated as μl. CO_2/mg. dry wt.

was some difference in the relative activities of the four corticoids studied as compared with those found for rat mammary gland slices. These inhibitory effects in sheep tissue must surely indicate that the corticoids do not act merely as insulin antagonists, for sheep tissue does not appear to be sensitive to insulin (Balmain & Folley, 1951; Balmain *et al.* 1952a). The results on the rat, therefore, as far as they go, are in harmony with the known physiological actions of insulin and adrenal cortex steroids in the whole animal. It must be noted, however, that since relatively high concentrations of steroids were necessary in order to produce noticeable inhibitory effects on lipogenesis, it is not certain how far these results are of physiological significance. From what is known of the concentrations of adrenal corticoids in the blood stream, it seems that the concentrations used in these experiments were very much higher, possibly of the order of a thousand times. On the other hand, it must be remembered that conditions in vitro and in vivo are very different and it would perhaps be premature to say that these results have no physiological significance.

d. Other Hormones

In addition to its well-known diabetogenic properties, the metabolic actions of somatotrophin (STH) include effects on lipid metabolism (see review by Ketterer, Randle & Young, 1957). We therefore considered it of interest to see whether STH added in vitro to rat mammary slices would affect the rate of lipogenesis in the slices, as indicated by respiration

measurements. In one series of experiments, cited by Folley (1955), definite although small increases in R.Q. were obtained with one purified preparation of STH (Armour M308; 100 μg. STH/ml. of medium). In later experiments with this and other preparations, such stimulatory effects could not be regularly obtained. We are left therefore with the impression that STH has no marked effects on lipogenesis in vitro in mammary tissue. It may be noted that Ottaway & Bulbrook (1955) have reported that, according to concentrations used, STH added in vitro will depress or increase the glucose uptake of rat diaphragm.

Direct effects of another pituitary hormone, intermedin, on the metabolism of lactating rat mammary gland slices have been described by Bradley, Folley, Landgrebe & Mitchell (1954) and Bradley & Mitchell (1957). These workers found that highly purified preparations of intermedin significantly increased the net gas output and the glucose uptake of this tissue. It seems possible that these effects may be connected with changes in the rate of lipogenesis.

Another endocrine gland which is known to affect fat metabolism, especially the synthesis of milk-fat, is the thyroid gland (see review by Blaxter, 1952). We have, therefore, studied the effects of triiodothyronine and triiodothyroacetic acid on the metabolism of mammary tissue in vitro (Folley and McNaught, unpublished work). In the case of triiodothyronine, 5 μg./ml. of the medium had no effect on the incorporation of labelled acetate into the fatty acids of sheep mammary gland slices. Triiodothyroacetic acid at concentrations of less than 10 μg./ml. had no effect, but, at higher concentrations, a depressing effect on the R.Q. of rat mammary gland slices, thus suggesting a possible inhibition of lipogenesis. The decrease in R.Q. was not brought about by increased oxygen uptake, as would be expected if the action of triiodothyroacetic acid was to uncouple oxidative phosphorylation. We know of only one instance (Thibault & Pitt-Rivers, 1955) of a thyroid active compound (triiodothyroacetic acid) having an immediate stimulatory effect in vitro on the respiration of mammalian tissue (kidney from thyroidectomized rats). This does not appear to have been confirmed (Pitt-Rivers, 1958).

In conclusion it must be admitted that insulin is the only hormone so far found to exert marked and reproducible effects on lipogenesis in vitro in relatively minute concentrations. The question might be asked whether this is because comparatively large molecules, like those of steroids and more particularly of proteins, under in-vitro conditions, are unable to enter the cell. However, the fact that the only regular effect has been obtained with insulin, a comparatively large molecule, would argue against this hypothesis or perhaps indicate that the biological effect under discussion does not depend on entry into the cell. Furthermore, investigation of preparations of broken cells and isolated enzyme systems has, so far, failed to demonstrate the mode of action of hormones on metabolism. It may well be, as Pitt-Rivers (1958) suggests, that "more physiologically integrated systems" will have to be investigated.

The question may also be asked how far these studies on fat synthesis in mammary tissue are relevant to the problem of fat synthesis in the body as a whole, for milk-fat is a highly specialized and rather atypical fat. Recent work on the biosynthesis of fats has tended to show that both liver and mammary gland fats, different though the final products may be as regards fatty acid composition, seem to be largely synthesized from two-carbon units by similar mechanisms (see Green, 1956). It seems justifiable, therefore, to conclude that information obtained from studies of the effect of hormones on lipogenesis in the mammary gland will be applicable to other tissues.

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FAT ABSORPTION AND ITS DISORDERS

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1. Mechanism of Fat Absorption in the Rat

During the last twenty years fat absorption has been more extensively studied in the rat than in any other experimental animal. The information obtained from these studies forms the basis for the generally accepted view of the fat absorption mechanism.

In the upper intestinal lumen, pancreatic juice and bile are clearly important, since the absence of either of them results in defective fat absorption. The components that appear to be necessary are pancreatic lipase and bile salts, although their replacement in the deficient state does not entirely restore fat absorption to normal. Until 1943, pancreatic lipase was thought to cause rapid and complete hydrolysis of glycerides to fatty acids and glycerol. It was shown, however, that pancreatic lipase causes the formation of mono- and di-glycerides and fatty acids, with a relatively slow liberation of free glycerol both in vitro and in vivo (Frazer & Sammons, 1945). This was confirmed by Desnuelle, Naudet & Rouzier (1948); Desnuelle, Naudet & Constantin (1950, 1951) and Mattson, Benedict, Martin & Beck (1952). It has been shown recently that other lipases, such as lipoprotein lipase (clearing factor), also give rise to mono- and di-glycerides during lipolysis (Carlson & Wadström, 1957).

Since the work of Pflüger (1901) complete pancreatic lipolysis had been regarded as an obligatory step in glyceride absorption, since by this means the fatty acids and glycerol could be brought into aqueous solution (Verzár & McDougall, 1936; Bloor, 1943). However, as a result of many experimental studies, in vitro, in animals and in man, it was concluded that complete pancreatic lipolysis of glycerides to fatty acids and glycerol was not a necessary step in their absorption and that glycerides could be absorbed from the intestinal lumen as such (Frazer, 1943a, 1943b, 1948). This view was confirmed by Favarger & Collet (1949), Favarger, Collet & Cherbuliez (1951), using deuterium-labelled fats and glycerol; by Karnovsky & Gidez (1951) and Reiser, Bryson, Carr & Kuiken (1952) with ^{14}C-labelled materials; and by Blomstrand, Tryding & Westöo (1956) who report an ingenious experiment using dimethyl stearic acid.

Realization that lipolysis need not be complete raised new problems in the fat absorption field. The existence of fine intraluminar emulsification had been known for nearly a century. The products of partial lipolysis, fatty acids and lower glycerides, together with bile salts, were shown to provide an emulsifying system that was effective under the conditions prevailing in the intestinal lumen (Frazer, Schulman & Stewart, 1944). The only function attributed to this fine emulsification was the facilitation of rapid and complete hydrolysis. It was, therefore, of interest to determine whether fine emulsification of the water-insoluble glyceride fraction represented an important step in its absorption, apart from any facilitating effect emulsification might have upon the rate and extent of hydrolysis.

The absorption of unhydrolysable materials, such as paraffins, had been previously reported and was known to occur in small quantities (Channon & Collinson, 1929; Channon & Devine, 1934). However, the mechanism of its absorption had not been studied. Finely emulsified paraffin introduced into the lumen of the small intestine was shown to be absorbed, provided that the particles were less than 0.5 μ in diameter (Frazer *et al.* 1944). Subsequently, the importance of an emulsifiable oil vehicle for the absorption of paraffin or lipid-soluble unhydrolysable esters was demonstrated in both short-term and long-term experiments. The presence of olive-oil increased the absorption of paraffin to 60%, whilst the addition of tributyrin, which is not emulsified in the intestinal lumen, did not facilitate paraffin absorption. The absorption of the paraffin in these studies has been checked by recovery from the chyle and the carcass and by the use of ^{14}C-labelled hexadecane. Emulsification to a particle size of less than 0.5 μ diameter was again found to be a necessary condition for absorption (Frazer, 1951; Frazer, Pover & Sammons, 1954). These observations on the need for an oil vehicle and fine emulsification for the absorption of paraffin have been confirmed by Shoshkes, Geyer & Stare (1950) and Bernhard & Scheitlin (1952).

Why should fine emulsification be an important step in the absorption of the water-insoluble fraction? For many years the absorbing cell of the small intestine has been known to have an outer striated border, the structure of which has been the subject of some controversy. In 1942, Baker described fine spindle-shaped canals penetrating this outer border. These were demonstrated in a wide range of preparations from many different species. Later, Baker (1951) also showed that spindle-shaped bodies which stain with Sudan Black could be seen in this outer border during absorption of butter-fat, but not during the absorption of non-lipid materials. These structures closely resembled in position, distribution and appearance the canals which he had described earlier. These findings have been confirmed by several workers (Frazer *et al.* 1954; Hewitt, 1954; McNaught, 1955).

We have made one further observation on these spindle-shaped bodies, which is of some interest. If olive-oil was fed, instead of butter-fat, spindles staining with Sudan Black could not be demonstrated in the outer border of the intestinal cells; if, however, a small amount of phosphatidylcholine was added to the olive-oil, the spindles of fatty material stained readily. This indicates that significant amounts of phosphatidylcholine were not formed in the intestinal lumen from olive-oil in these experiments; absorption was, however, effective without the addition of lecithin.

Electron-microscope studies have revealed that the solid part of the striated border of the intestinal cell consists of finger-like processes or microvilli (Granger & Baker, 1950).

Since the sections for electron microscopy were about 0.1 μ thick, it is, perhaps, not surprising that Baker's canals, which are 0.3 μ in diameter, were not readily demonstrated in such preparations. There seems to be no fundamental reason why both these structures should not co-exist. Palay (personal communication, 1956) demonstrated fat particles passing between microvilli and he considers that the particles are then taken up into the cell by pinocytosis. The existence of the microvilli and canals makes fine dispersion of the water-insoluble fraction an obligatory step in its absorption, since the fatty material cannot gain access to the cell in the form of large droplets. The critical size of dispersion necessary for penetration into the outer border and subsequent absorption will clearly depend upon the size of the spaces between the microvilli. From electron-microscope studies, it appears that penetration between the microvilli would require a dispersion to a particle size of the order of 0.1 μ or less. Available evidence suggests that Baker's canals—as well as the microvillous structure—may be present as functional units. If Baker's canals exist, emulsification to a particle size of rather less than 0.5 μ would suffice.

If the fine emulsion penetrating into the outer border of the intestinal cell was compared with that found in the intestinal lymph, certain differences were evident. The particles leaving the lumen were essentially a mixture of tri-, di- and monoglycerides and fatty acids. The emulsion was stabilized by a system which included fatty acids, lower glycerides and bile salts. The stability of the emulsion particles was not affected by D-lecithinase, and flocculation of the particles occurred in the presence of plasma proteins at pH 7.4. The flocculation pattern resembled that seen with simple, negatively charged emulsions. The fat particles in the intestinal lymph, on the other hand, contained long-chain triglycerides; lower glycerides and short-chain fatty acids had disappeared from the lipid phase; the other principal constituent was phosphatidylcholine, which might account for as much as 6% of the lipid. These emulsion particles did not flocculate in the presence of plasma protein; indeed, plasma protein appeared to be concerned with their stability and properties. The addition of D-lecithinase resulted in rapid flocculation and creaming (Frazer, 1949b).

It has been implicit in all concepts of fat absorption put forward during the present century that the intestinal cells are metabolically active units. Since a mixture of glycerides entered the intestinal cell, while almost all the long-chain fat absorbed could be recovered as triglycerides in the chyle, it was obvious that synthesis must occur during absorption. Furthermore, the formation of phospholipids in the intestinal cells was firmly established by 1936 (Verzár & McDougall, 1936). This metabolic activity has been emphasized by recent work with isotope-labelled materials (Bloom, Chaikoff, Reinhardt, Entenman & Dauben, 1950; Bergström, Borgström, Carlsten & Rottenberg, 1950; Bergström, Blomstrand & Borgström, 1954).

Long-chain fatty acids might be reincorporated into glycerides, but fatty acids with less than ten carbons were not. From indirect evidence, it was suggested that these short-chain fatty acids might pass into the body by the alternative portal route, while the water-insoluble fraction passed in the chyle (Frazer, 1946). Direct evidence, using labelled short-chain fatty acids, has since been obtained in support of this concept (Kiyasu, Bloom & Chaikoff, 1952). It was also shown that labelled free glycerol was not readily taken up into glycerides in the intestinal mucosa (Favarger, 1950; Karnovsky & Gidez, 1951; Reiser *et al.* 1952).

The formation of phospholipids during fat absorption was demonstrated by Sinclair (1929, 1936), Artom & Peretti (1935) and Hahn & Hevesy (1938). These investigators established that the phospholipid molecules could be formed in the intestinal mucosa from dietary constituents. Sinclair (1936) considered that phospholipid was a necessary step in the resynthesis of glycerides from fatty acids and glycerol. Schmidt-Nielsen (1946) and Zilversmit, Entenman & Chaikoff (1948) showed that phosphorus turnover in the intestinal cells was not necessarily altered during fat absorption. It was therefore concluded that phospholipid was not an obligatory intermediate in the absorption of glycerides.

Nevertheless, phospholipid does seem to be concerned in some way with the mechanism of fat absorption. Both lecithin (Augur, Rollman & Deuel, 1947) and glycerophosphate (Verzár & Laszt, 1934) caused improvement in fat absorption. We confirmed these observations and also demonstrated that addition of choline caused a significant increase in the amount of glyceride absorbed in three hours. In studies carried out by my colleague, P. E. Sagrott (1948), the rate of absorption was increased 37% by 120 mg. of lecithin, 63% by 10 mg. of glycerophosphate and 52% by 10 mg. of choline (Frazer *et al.* 1954). These effects are still unexplained.

Consideration of these points leads to the conclusion that phospholipid is added to the lipid particles in the course of their absorption through the intestinal mucosa. Phospholipid is an important and apparently necessary link between the glyceride mass and the outer protein film. If this outer film is derived from the intestinal cell, as seems likely, phospholipid formation in the intestinal cell may be a necessary step to provide this, or for the replacement of materials in the intestinal cell that are used for this purpose. It is possible that protein synthesis may also be necessary. If this is so, limitation of phospholipid formation or protein synthesis, or both, in the intestinal cell should affect particulate fat absorption. We have made preliminary studies which strongly indicate that this is the case. If this is so, it would explain how a small amount of phospholipid can facilitate glyceride absorption and why phospholipid turnover in the intestinal cell need not necessarily increase during fat absorption, since preformed phospholipid, either in the diet or in the bile, might satisfactorily fulfil the function required.

It has been claimed that the adrenal cortex plays a vital part in the fat absorption mechanism. Double adrenalectomy in the rat caused depression of absorption of long-chain fats (Verzár & Laszt, 1935). Short-chain glycerides were normally absorbed, however (Bavetta & Deuel, 1942). Salt therapy restored the absorption of long-chain fats almost to normal levels (Barnes, Miller & Burr, 1941). It seems probable that the effect of double adrenalectomy is essentially secondary to water and electrolyte changes.

The visible particulate fatty material leaving the intestinal cell appears to pass selectively into the lacteals and thence into the chyle. Short-chain fatty acids, and perhaps under certain circumstances long-chain fatty acids, either in aqueous solution or in a state of micellar dispersion, penetrate successfully into the blood capillaries and can be transported in the portal blood. Under the circumstances of normal glyceride absorption, the bulk of absorbed fat passes in the chyle into the systemic blood, causing a demonstrable hyperlipaemia. The quickest way of studying this hyperlipaemia is by means of the

chylomicrograph technique (Frazer & Stewart, 1939). As with all tests of this type, the assessment of absorptive rate depends upon an assumption that the rate of removal does not alter greatly during the course of the experiment. For the simple purposes for which it was devised, the chylomicrograph technique is satisfactory.

Extensive studies on the structure of the chylomicron showed that it consisted of a glyceride core, with an outer film of phospholipid and protein. The phospholipid appeared to be mainly phosphatidylcholine, and the protein was β-globulin. The majority of particles ranged in diameter from 350 Å up to 0.5 μ, but occasional particles were seen that were 1 μ or more in diameter. The smaller particles appeared dull under dark-ground illumination, as compared with the larger bright particles. During alimentary hyperlipaemia both dull and bright particles are greatly increased (Frazer & Stewart, 1937; Elkes, Frazer & Stewart, 1939).

Thus, the full picture of the fat absorption mechanism in the rat is slowly evolving. Is it applicable to man? Recent studies on some of the common disorders of fat absorption in human subjects provide an opportunity for re-examination of this question.

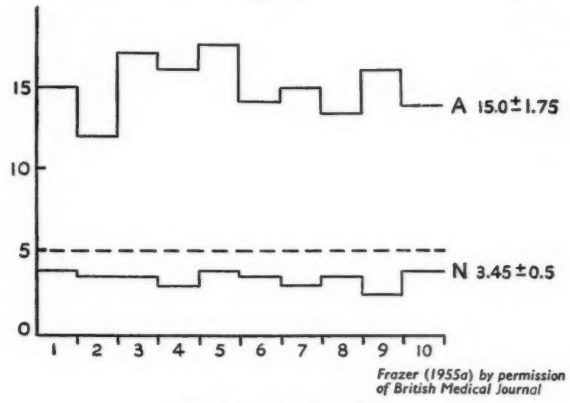
2. Origin and Significance of Human Faecal Fat

The daily long-chain fatty acid content of normal human stools is rather less than 5 g. Difference between intake and output indicated that fat absorption was normally greater than 95% on a fat intake of 50–150 g. However, if labelled fats were used, the level of assimilation of those that were liquid at body-temperature was of the order of 97–99%. If the melting-point of the fat was higher than body-temperature, absorption might be less complete (Cheng, Morehouse & Deuel, 1949), but it could be improved by the addition of a vehicle of liquid fat (Mattil & Higgins, 1945). Progressive hydrogenation of a liquid oil caused increasing interference with absorption, presumably for the same reason (Holmes & Deuel, 1921).

Levels of faecal fat were used to determine whether fat absorption was normal. It was not necessary to have an accurate measurement of intake, provided that it was within the range of 50–150 g. and did not contain an excess of long-chain saturated fatty acids. If, on a normal hospital diet, the faecal fat was maintained over five days or more at a mean daily level of less than 5 g., no fat absorption defect was present. If this level was exceeded, a fat absorption defect might be present. This increase of long-chain faecal fat was the cardinal change in steatorrhoea (Frazer, 1955a). The increase was often considerable, as shown in fig. 1. The additional faecal fat might be directly derived from unabsorbed dietary fat. This is termed the "dietary component". In patients with pancreatic enzyme deficiency and in most patients with enteropathy, there was a significant increase in the dietary component, and this might be the major contribution to the increase of faecal fat.

An alternative source of increased fat was by synthesis from short-chain compounds, or from excretion into the intestinal lumen. This is termed the "non-dietary component", although the fat may be derived from dietary constituents other than fat. Short-chain compounds suitable for long-chain fatty acid synthesis might be formed in large quantities as a result of carbohydrate fermentation. There were indications that a moderate increase in the non-dietary component commonly occurred in steatorrhoea. In some patients with chronic sprue, marked steatorrhoea has been observed which was out of proportion to the demonstrable degree of enteropathy. In these patients, it seemed possible that the non-dietary component made a major contribution to the additional faecal fat. Even more interesting was the fact that the nine patients so far studied have all shown a dramatic disappearance of the additional faecal fat following antibacterial therapy, as shown in fig. 6. It seems likely that this effect was due to the changes in intestinal flora caused by the treatment.

FIG. 1. LEVELS OF NORMAL AND ABNORMAL FAECAL FAT



Frazer (1955a) by permission of British Medical Journal

Abscissae: time (days)
Ordinates: faecal fat (g.)

A: abnormal faecal fat, and
N: normal faecal fat, both on a daily fat intake of 50 g./day

The figure on the right of each curve is the daily mean value (with standard deviation) for a 10-day period.

What is the mechanism by which a change in flora could cause such a dramatic reduction in the faecal fat? It is possible that active fermenting flora might cause hydrogenation of unsaturated dietary fats to such an extent that the amount of liquid oil vehicle present was inadequate to ensure their absorption. In order to alter the quantitative level of fat in the stools, these changes would have to occur in the lumen of the small intestine. If, however, the non-dietary component were increased, the flora might play a part by forming a large amount of short-chain fragments by fermentation, or by providing a site for synthesis. Alternatively, the actual synthetic process might occur in the intestinal wall, or even elsewhere. Apart from increased synthesis at an extra-intestinal site, with subsequent excretion into the intestinal lumen, it is difficult to see why modifications of the flora should affect fat excretion.

It is, perhaps, surprising that there are no methods readily available at the present time that will enable the origin of faecal fat, or even the proportions of the dietary and non-dietary components, to be determined in any individual patient. It is likely that techniques using labelled fat will eventually provide the answer.

3. Faulty Intraluminar Emulsification in Human Subjects

Pancreatic enzyme deficiency is one of the common causes of steatorrhoea. This may arise from diseases affecting the

pancreas, from obstructive lesions of the pancreatic ducts, from protein insufficiency, or, in rare cases, from a specific deficiency of pancreatic lipase. In many of these patients the enzyme defect is permanent.

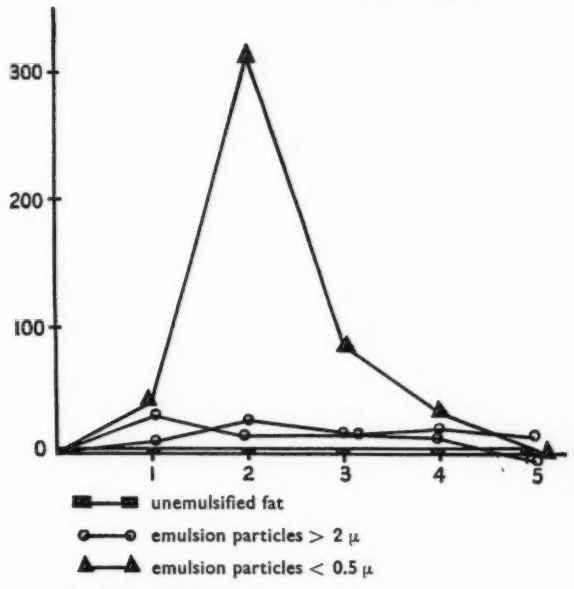
The absence of pancreatic lipase must be established by intubation; the degree of hydrolysis of fat in the stools may be quite misleading. If ingested fat was recovered by intestinal intubation, emulsification in the upper intestinal lumen was shown to be defective. The addition of lipase to the samples, and subsequent agitation, caused rapid emulsification in vitro. Function of the upper small intestine, as shown by absorption tests with water-soluble substances, radiography and biopsy, was usually normal in these patients. The chylomicrograph, however, was characteristically flat (Frazer, 1952a).

If the concept of the significance of fine intraluminar emulsification derived from the studies in rats is correct, the reason for the lack of particulate absorption is difficulty of access of the fatty material to the intestinal cell; the water-soluble fraction is unable to penetrate into the outer border. A simple investigation was carried out to test this hypothesis. Finely emulsified fat was administered, either by tube, or, in the case of infants, in a suitable buffer medium, to patients lacking pancreatic enzymes and with normal upper intestinal absorptive capacity. If the particles were less than 0.5 μ diameter, a dramatic systemic hyperlipaemia occurred. In a group of eight children with deficiency of pancreatic lipase, the average increment in fat particles in the systemic blood after ingestion of unemulsified fat was 21 (range 4–62), and with emulsified fat it was 144 (range 65–315). The normal increment was 150. The differences between these two groups were highly significant statistically (Frazer, 1955b). One such study was carried out by Sheldon (1955), with material supplied by us, in a child with a specific pancreatic lipase deficiency; the result is shown in fig. 2. An emulsion of 2 μ particle size, which would greatly facilitate lipolysis, caused no change in the systemic hyperlipaemia in this patient.

These studies confirm the importance of pancreatic lipase in man and the significance of fine emulsification for the absorption of the water-insoluble fraction under circumstances in which facilitation of lipolysis did not arise.

Bile deficiency also caused interference with fat absorption, but most of the cases were too complex to permit detailed study of the precise effect on fat absorption. There seems little doubt, however, that, as in the rat, bile is important for the absorption of fat in man.

FIG. 2. EFFECT OF ADMINISTRATION OF FAT TO A PATIENT WITH PANCREATIC LIPASE DEFICIENCY



Unemulsified fat, or emulsions containing particles of 2 μ diameter, had no effect; finely emulsified fat with particles of less than 0.5 μ diameter caused a normal hyperlipaemic response.

4. Upper Intestinal Enteropathies

The conditions known as the coeliac syndrome, idiopathic steatorrhoea and sprue have provided much opportunity for speculation on the mechanism of fat absorption in man. The fat absorption fault which is a common feature in all of them has been variously ascribed to infection or infestation, to metabolic disorders such as adrenal insufficiency, electrolyte disturbances, or faulty phosphorylation, or to dietary factors such as vitamin deficiencies, inability to handle wheat flour, or ingestion of rancid fats. Recent studies have advanced knowledge sufficiently to enable us to place most of these earlier concepts in their proper perspective.

The great majority of patients with the coeliac syndrome and idiopathic steatorrhoea suffer from a gluten-induced enteropathy, which can be distinguished on pathogenic grounds from the enteropathy of sprue. Only these two forms of enteropathy will be considered, since the others add little to our general understanding of fat absorption at the present time.

a. Gluten-Induced Enteropathy

Diagnosis. In a typical patient faecal fat was significantly increased, commonly to 10–20 g. daily—levels being rather less than those observed in pancreatic enzyme deficiency. The fat was present mainly as long-chain saturated fatty acids, which might form sheaves of crystals in the stools. The motions were usually bulky, pale, fluid or semi-fluid, foul and fatty, but, occasionally, there was no change other than an increase of long-chain fats, and even this abnormality might be discovered only on analysis. These alterations in the nature of the stools were largely due to the effects of intestinal flora which flourished owing to the presence of unabsorbed food materials in the lower reaches of the intestine.

The pancreatic enzymes and bile salts were within normal limits. The upper intestinal enteropathy could be characterized by the application of three sets of tests, as shown in Table I. Measurement of absorption rate showed that absorption of many water-soluble and lipid-soluble substances was depressed and delayed. Radiography revealed a clumping pattern with simple barium sulphate suspension; a non-flocculable barium sulphate suspension, such as Raybar, revealed dilatation and a " stack of coins " pattern, instead of the normal feathery appearance (Frazer, 1952b). Biopsy may show various abnormalities, commonly shortening of villi and

TABLE I. DIFFERENTIAL DIAGNOSIS OF PANCREATOGENOUS AND ENTEROGENOUS GROUPS IN PATIENTS WITH STEATORRHOEA

The hepatogenous group resembles the pancreatogenous, except that pancreatic enzymes are present, but bile salts are deficient

	Pancreatogenous group	Enterogenous group (upper intestinal lesion)
Daily mean faecal fat (g.)	Commonly 20–50 or more	Commonly 10–20
Pancreatic enzymes	Deficient	Normal range
Absorption rate:		
Water-soluble substances	Normal	Delayed and depressed
Lipids	Markedly depressed	Delayed and depressed
Radiography:		
Flocculable medium	Normal	Clumped pattern
Non-flocculable medium	Normal	Dilatation; "stack of coins" pattern
Intestinal biopsy	Normal	Abnormal

increased cellular infiltration; in more severe cases, the villous structure may be grossly disorganized (Frazer, 1955a, 1956).

Aetiology. Intestinal infection has been thought to play a part in the initiation of coeliac disease in the infant; this may be so. Infestation, especially with *Giardia*, may lead to a syndrome that is in many ways similar, but it clears up dramatically on treatment with mepacrine, which has no effect on gluten-induced enteropathy. There has been no serious suggestion that infection plays any significant part in the maintenance of the coeliac syndrome.

Causation by metabolic disorders has been strongly supported in the past. Verzár (1935) put forward the suggestion that the condition was caused by adrenal insufficiency. It is quite clear, however, that these patients do not suffer from primary adrenal insufficiency, as it is also evident that those with Addison's disease do not have gluten-induced enteropathy, or any other sprue-like syndrome. Cortisone administration may cause some improvement, which is maintained only so long as cortisone therapy is continued. This is probably due to the well-known effect of cortisone on tissue response to injury.

Electrolyte disturbances are common in patients with continuing and severe diarrhoea. Electrolyte imbalance may well cause depression of function of the small intestine, which will be superimposed on the original cause of the diarrhoea. Imbalance should be corrected when it occurs; its correction will not affect an underlying gluten-induced enteropathy.

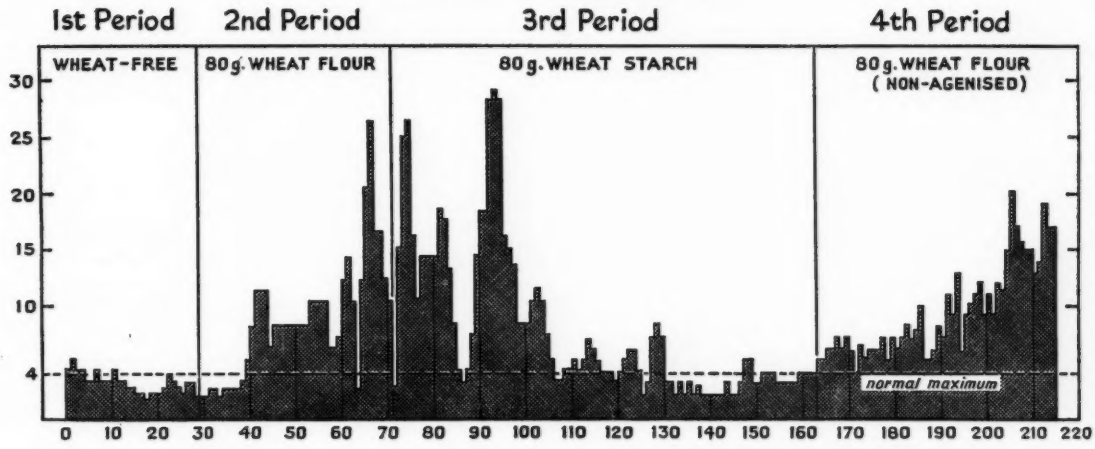
The main evidence for a specific phosphorylation fault was based on factually wrong information. It was thought that only the absorption of certain substances, such as glucose, was depressed. It is now known that there is a general depression of absorption. There was an almost identical depression of the absorption of glucose and urea in a series of ten patients with idiopathic steatorrhoea (Frazer, French, Thomas & Thompson, 1952). The selective deficiencies of riboflavine and nicotinic acid, that are sometimes seen, may be explained on different grounds (Frazer, 1949a). The concept of a specific phosphorylation fault has, therefore, not been substantiated.

Of the dietary factors, vitamin deficiencies can be shown to be secondary to the primary fault. They usually involve folic acid, riboflavine and nicotinic acid. They are a relatively late development and show considerable variability. The deficiency states can be corrected by appropriate therapy without materially affecting the steatorrhoea. The clumping pattern so commonly seen in the small intestine in these patients was ascribed to vitamin deficiencies by Golden (1945); we were able to show, however, that the change was due to flocculation of the barium sulphate by excessive mucous secretion (Frazer, French & Thompson, 1949).

The deleterious effect of wheat flour in children with the coeliac syndrome has been appreciated for some time. Andersen (1947) and Sheldon (1949) considered that this effect was due to the wheat starch. Van de Kamer, Weyers & Dicke (1953) were able to show, however, that the deleterious effect was due to the 10% of protein rather than the 90% of starch present in the wheat flour. This basic observation was soon confirmed (Anderson, Frazer, French, Gerrard, Sammons & Smellie, 1952; Sheldon & Lawson, 1952). We demonstrated that wheat gluten was the cause of the enteropathy found in the great majority of children with the coeliac syndrome. Removal of wheat gluten from the diet allowed the intestine to recover and the patient to return to normal; reintroduction of wheat gluten caused a return of the enteropathy and the signs and symptoms of coeliac disease (fig. 3) (Ross, Frazer, French, Gerrard, Sammons & Smellie, 1955). It was soon found that many adult patients with idiopathic steatorrhoea also benefited from a gluten-free regime (Anderson, Frazer, French, Hawkins, Ross & Sammons, 1954). However, we do not accept a diagnosis of gluten-induced enteropathy merely on the basis of improvement on a gluten-free regime, even if the patient returns to normal. We insist upon a definitive test of gluten sensitivity. Gluten is reintroduced into the diet and the faecal fat level is studied. An unequivocal increase of faecal fat to abnormal levels during this feeding period is taken as confirmation that the patient is suffering from gluten-induced enteropathy (fig. 4). It is clear that these patients do not handle wheat gluten effectively and an enteropathy results. This can be completely relieved in children, and in patients with a relatively short history, by the complete exclusion of wheat gluten from the diet; even adults, who have had severe steatorrhoea for many years, may show startling improvement on a gluten-free regime.

What does wheat gluten do in these patients? Apart from wheat and rye gluten, these patients can usually eat anything. Complete acid hydrolysis of wheat gluten renders it harmless; so does deamidation. Enzymic degradation with pepsin and trypsin, however, does not materially reduce toxicity. From this enzymic hydrolysate an autoclaved peptide fraction can be prepared which readily causes deterioration in these patients. Further digestion of this fraction with an extract of the mucosa of the hog's intestine appears to make it innocuous. A detailed study of the mechanism of this effect is being undertaken at the present time by my colleague, B. Shaw, in our laboratory. Berger (1958) suggests that gliadin causes an antigen-antibody reaction and that children with the coeliac syndrome can be distinguished from normal

FIG. 3. FAECAL FAT LEVELS IN A PATIENT WITH GLUTEN-INDUCED ENTEROPATHY UNDER VARIOUS DIETARY CONDITIONS



Anderson, Frazer, French, Gerrard, Sammons & Smellie (1952) by permission of Lancet

Abscissae: time (days)
Ordinates: faecal fat (g./day)
First period: gluten-free regime
Second period: gluten reintroduced
Third period: wheat starch continued, but wheat gluten withdrawn
Fourth period: wheat gluten reintroduced

Remission occurred in the first and third periods; deterioration occurred in the second and fourth periods.

children by a complement-fixation test. If this is so, the peptide fraction must be able to engender this antigen-antibody response. Alternatively, a direct damaging or pharmacological effect by the peptide or peptides must occur; attempts to block the action of gluten or gluten fractions with antihistaminic agents have so far been unsuccessful in these patients.

It may be concluded that the fat absorption fault in gluten-induced enteropathy is due to a generalized interference with the absorptive capacity of the upper small intestine by wheat gluten in a group of people who seem to handle this protein differently from the majority of other human subjects. It may, perhaps, be added that not only do the enteropathy and the steatorrhoea clear up on a gluten-free regime in these patients, but that the anaemia associated with folic acid deficiency, if present, may show spontaneous remission and growth may occur at a greater than normal rate in patients with dwarfism (Frazer, Fletcher, Sammons & Williams, 1958; Frazer, 1956).

b. Sprue

Sprue is an entirely different proposition. It has, in the first place, a peculiar geographical distribution, whereas gluten-induced enteropathy is almost ubiquitous. Sprue is seen in the Far East, India, and the Caribbean, but it is rare in Africa. Even in the endemic areas it has a patchy distribution. Thus, at the present time it is common in Hong Kong, but rare in Singapore; it probably still occurs in India, but it is said to have disappeared from Ceylon. In the Caribbean, it is wide spread in Puerto Rico, but almost unknown in Jamaica.

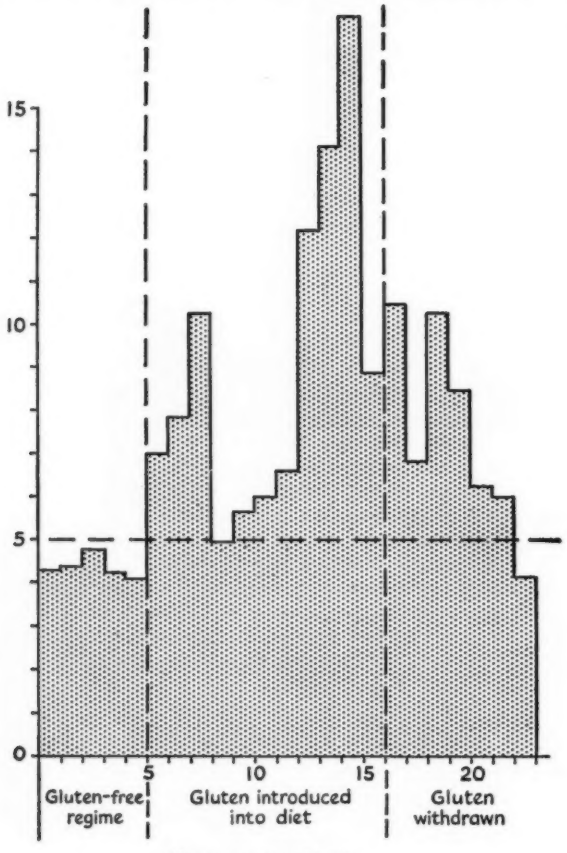
Diagnosis. The type of sprue that we have been able to study occurs in young healthy European adults in Hong Kong. It may develop within a few weeks or months of arrival in the area. The patient develops severe lassitude, loss of appetite and loss of weight. As this develops, diarrhoea starts and the patient passes pale, fluid, fatty, offensive stools. Examination reveals an enteropathy with the same general features as those described for gluten-induced enteropathy. At this stage the blood may be normal, but sore tongue or buccal ulceration is common. Later an anaemia, typical of folic acid deficiency, usually develops (Frazer, 1952b).

Aetiology. Infection has been thought to play a part, but there is little clear evidence to support this contention. It is possible that a severe infection of the bowel might predispose to the development of sprue, but it has not been a feature of the patients we have studied.

The usual group of metabolic disorders have been suggested. Adrenal insufficiency has been excluded by appropriate investigation (Panigua, Casas & Hernández-Morales, 1950). Electrolyte imbalance might obviously contribute to the enteropathy in some patients, but must be regarded as essentially secondary. Faulty phosphorylation might be involved as part of a wider metabolic disturbance; the same general arguments already discussed in connexion with gluten-induced enteropathy seem to apply in sprue; the depression of absorption appears to be generalized rather than specific, although in sprue this point may not have been so fully studied.

On the dietary side, gluten sensitivity can be excluded; no effect was observed when gluten was excluded, or when it was reintroduced into the diet. Folic acid deficiency is different

FIG. 4. DEFINITIVE TEST FOR GLUTEN SENSITIVITY



Abscissae: time (days)
Ordinates: faecal fat (g./day)

The effect of reintroduction of gluten into the diet of a patient with gluten-induced enteropathy in whom faecal fats had been reduced to normal levels on a gluten-free regime.

in sprue from that seen in gluten-induced enteropathy. Whereas in the gluten-sensitive patient folic acid deficiency was a late feature, of mild degree, and could be corrected without affecting the steatorrhoea, in sprue it was seen early, it was severe, and, in the acute case, treatment in hospital and parenteral folic acid therapy caused definite improvement of the enteropathy and steatorrhoea, as shown in Table II. It is perhaps difficult to decide how much each of these two treatments contributed to the improvement. However, we have recently had a patient who developed acute sprue just after returning to Great Britain from Hong Kong. He had an acute and severe folic acid deficiency and all the classical features of sprue. Parenteral folic acid therapy caused a striking decrease in faecal fat, in spite of increased food intake (fig. 5).

This observation prompted us to examine further the effect of folic acid on fat absorption in the rat. In animals made deficient in folic acid by the method of Kodicek & Carpenter (1950), we had been unable to demonstrate any significant

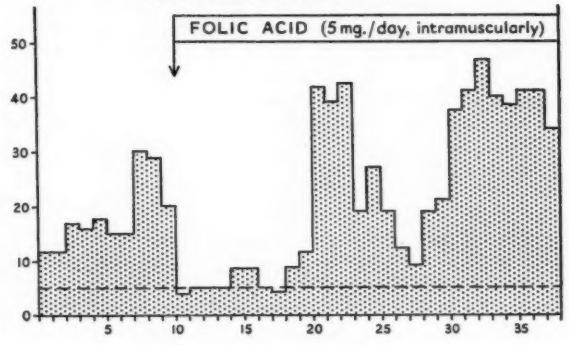
TABLE II. IMPROVEMENT IN TEN PATIENTS WITH SPRUE, FOLLOWING TREATMENT IN HOSPITAL AND PARENTERAL FOLIC ACID THERAPY (15 mg. daily for 15 days) (S. A. Biggart, personal communication, 1954)

The range of values obtained is shown in parenthesis

	Before	After	Normal
Mean daily faecal fat (g.)	18 (10–24)	7 (5–15)	<5
Glucose absorption: Maximum increment (mg./100 ml.)	17 (7–30)	50 (29–90)	>40
Chylomicrograph: Maximum increment in number of particles/ microscopic field	65 (30–127)	120 (70–180)	150
Radiography Number of cases:			
Grossly abnormal	6	0	0
Abnormal	4	4	0
Normal or near normal	0	6	10

effect of the deficiency state on the absorption of either fat or xylose. This finding was in agreement with Woodruff (1950, 1952). It seemed to us, however, that the folic acid deficiency might not be sufficiently severe in these animals. We have, therefore, studied fat absorption in animals treated with aminopterin, which blocks the conversion of folic to folinic acid. Aminopterin intoxication has a dramatic depressant effect on fat absorption. The full implications of this are still under investigation, but it leaves no room for doubt that interference with folic to folinic acid conversion caused gross interference with water and fat absorption in rats. It is also

FIG. 5. THE EFFECT OF PARENTERAL FOLIC ACID IN A PATIENT WITH SPRUE



Abscissae: time (days)
Ordinates: faecal fat (g./day)

The immediate effect of folic acid injections was a marked reduction in faecal fat; with continued folic acid therapy the faecal fat increased to higher levels.

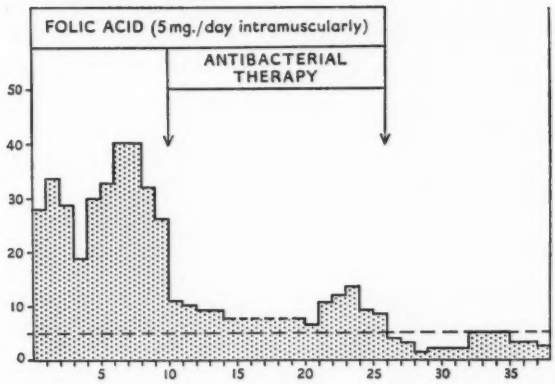
known that aminopterin intoxication markedly decreased the turnover of "phosphoprotein" in the intestinal cells of the rat (Vitale, Gerschoff, Sinisterra, Hegsted & Zamchek, 1956). Why should healthy young adults develop an acute and severe folic acid deficiency? The folic acid content of the diet appeared to be adequate. It seems almost inescapable that some factor must have been present that interfered with the utilization of dietary folic acid.

It has often been suggested that there might be some association between sprue and the ingestion of rancid fats. In areas of endemic sprue unsaturated vegetable fats are extensively used for frying. It was shown that such fats in use for deep frying in Hong Kong had often undergone significant oxidative rancidity. Investigation showed that groups using fats that did not undergo oxidative rancidity did not develop sprue. Many patients with sprue have consumed significant amounts of oxidized fats during the period immediately preceding the onset of the disease. French (1949), working in our laboratory, studied the effect of oxidized fats on rats. He showed that a transient steatorrhoea developed on high dosage. He concluded that the peroxide content of the fat was not important and that other unspecified products were responsible for the effects observed. Greenberg & Frazer (1953) demonstrated an increased relative weight of the small intestine in animals consuming oxidized fats. However, it has not been possible so far to link these observations effectively with the aetiology of sprue. The problem is still under active investigation by our group. It may not be entirely irrelevant to this problem that the blood tocopherol levels have been shown to be low in sprue patients (Darby, Cherrington & Ruffin, 1946).

The present knowledge about sprue does not enable us to draw any conclusions with regard to the fat absorption mechanism. Nevertheless, it is possible that further study of this condition may reveal facts of the greatest interest and importance. There is some reason for thinking that the interference with fat absorption in sprue is of a different and, perhaps, rather more specific nature than that observed in gluten-induced enteropathy.

Many of these patients with acute sprue recover completely when removed from their local environment and treated parenterally with folic acid. Some, however, do not. Out of 21 sprue patients flown home to us in Birmingham, seven developed a persistent steatorrhoea, in spite of complete relief of all signs of folic acid deficiency. We have since had two more patients of this type. The last one was the patient already mentioned, who developed sprue on his return to Britain. This patient developed persistent steatorrhoea while under constant observation in our metabolic unit. It will be seen in fig. 5 that, after the initial dramatic improvement in fat absorption, a progressive increase of the faecal fat level occurred. After five weeks' treatment with folic acid, he had gained 18 kg. in weight, his blood showed a reduction of mean corpuscular volume from 116 μ^3 down to 93 μ^3, the ulcers had disappeared from his mouth, and urinary recovery of xylose had improved from 2.5% to 21%; 25% is normal in our laboratory. In spite of this improvement, his faecal fat increased to a daily mean of 30.6 g., which was nearly twice

FIG. 6. THE EFFECT OF ANTIBACTERIAL THERAPY ON PERSISTENT STEATORRHOEA IN A PATIENT WITH SPRUE, IN WHOM FOLIC ACID DEFICIENCY HAD BEEN CORRECTED



Abscissae: time (days)
Ordinates: faecal fat (g./day)

Faecal fat showed marked reduction to normal levels after start of antibacterial therapy. The improvement was maintained.

the level observed before folic acid therapy was started. He was then given three successive five-day courses of succinyl sulphathiazole (6 g./day), chloramphenicol (1.5 g./day) and of chlortetracycline (1.0 g./day). At the end of this fifteen-day period of antibacterial therapy, the faecal fat averaged 3.3 g. daily for the following ten days (fig. 6). He has remained normal ever since. Precisely similar results were obtained in the other eight patients with persistent steatorrhoea. Some possible implications of this effect in relation to fat absorption and faecal fat output have already been discussed.

While information on all aspects of fat absorption in man is by no means complete, it may be concluded that there are strong indications that the mechanism of fat absorption in man is closely similar to that observed in the rat. The intraluminar phase in both species appears to require partial lipolysis and fine emulsification as obligatory steps in particulate glyceride absorption. The dependence of the alimentary hyperlipaemia on the rapid assimilation of particulate fat also seems to be a common feature. The fat particles found in the chyle and in the blood have a similar structure in both man and rat, being stabilized by a phospholipid-containing lipoprotein complex. It seems likely that further study of phospholipid and protein synthesis in the intestinal cell and the possible links between this and folic acid metabolism in rats, and in patients with sprue, may throw further light on some of the outstanding problems in the cellular and distributive phases of fat absorption.

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BIOSYNTHESIS OF CHOLESTEROL

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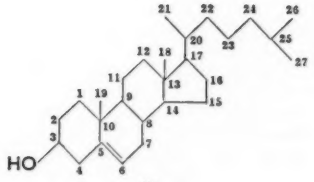
1 Acetate as a precursor of cholesterol
2 Squalene
3 Lanosterol
4 Earlier stages of biosynthesis
5 Mevalonic acid
6 Present state of knowledge
References

Cholesterol (formula I) is an alicyclic unsaturated alcohol present in all animal cells and in blood. It is the characteristic sterol of animals, the plant sterols having additional carbon in the side-chain. In the body, cholesterol functions as a precursor of the bile acids and of numerous hormones of the adrenals and gonads. Cholesterol itself is a constituent of lipoproteins and both in serum or in tissues may occur either free or esterified by fatty acids. During the past few years special attention has been drawn to the association of cholesterol with polyethenoid fatty acids and to the possible connexion of these esters with the pathogenesis of atherosclerosis (Bronte-Stewart, p. 243 of this Bulletin). The functions of cholesterol, apart from its role as a precursor, are probably related to the manner in which its molecules are oriented at a surface.

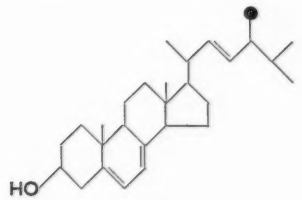


(I)

As well as being absorbed from food, cholesterol is synthesized in the animal body, as was shown quite early by feeding experiments. The liver is an important site of synthesis and appears to be the principal endogenous source of the cholesterol in blood plasma (Hotta & Chaikoff, 1955; Morris, Chaikoff, Felts, Abraham & Fansah, 1957). Many other tissues are also known to synthesize the sterol: the intestinal mucosa, ovary, and all foetal tissues of the rabbit (Popják & Beeckmans, 1950a, 1950b); the theca interna, theca externa, and zona granulosa of the hen's ovary (Popják & Tietz, 1953); the adrenal cortex, kidney, the testis and skin are all sites of active synthesis (Srere, Chaikoff & Dauben, 1948; Srere, Chaikoff, Treitman & Burstein, 1950). There is no evidence whether muscle (striated or smooth) or lymphoid tissue synthesize cholesterol or not. Brain tissue of the new-born animal is active in synthesizing cholesterol until myelination is complete; thereafter the process comes almost to a standstill (Waelsch, Sperry & Stoyanoff, 1940; Srere *et al.* 1950).

1. Acetate as a Precursor of Cholesterol

Though some early experiments can now be recognized as providing a clue to the mode of synthesis of cholesterol by the body, experimental techniques were not powerful enough to gain much insight into this process before the advent of isotopic labelling. In 1937 Sonderhoff & Thomas reported that yeast grown in sodium deuterioacetate (CD_3CO_2Na) solution accumulated a higher concentration of deuterium in the unsaponifiable portion of its fat than in any other of its constituents. This fraction is mainly ergosterol (II). Bloch & Rittenberg (1942a, 1942b, 1944) fed various substances labelled with deuterium to mice and rats, and found that only deuterioacetate (and substances transformed in the body to acetate) gave rise to significant incorporation of deuterium into cholesterol. Later (Bloch & Rittenberg, 1945; Bloch, Borek & Rittenberg, 1946), these results were confirmed by the use of acetate labelled with ^{13}C and ^{14}C; it was further shown that both carbon atoms of acetate were utilized, and from the results with acetate labelled at one of its two carbon atoms with ^{13}C and at the other with ^{14}C it appeared that the methyl carbon of acetate was incorporated into cholesterol to a greater extent than the carboxyl carbon, the ratio being approximately 5 : 4 (Little & Bloch, 1950).

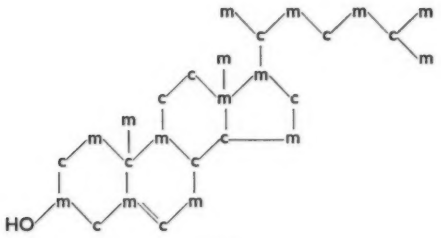


(II)

By chemical means, carbon atoms could be isolated from a few specified positions in the cholesterol molecule; and when these procedures were applied to cholesterol biosynthesized from [^{14}C]acetate (Little & Bloch, 1950) the results were illuminating. Positions in the cholesterol molecule were found to fall into two groups: when carboxyl-labelled acetate was the precursor the first group contained radioactive carbon and the other group contained none; when methyl-labelled acetate was the precursor the second group became labelled with ^{14}C and the first group showed only such feeble radioactivity as could be attributed to partial conversion of $^{14}CH_3CO_2H$ to $CH_3{}^{14}CO_2H$ in the body by operation of the tricarboxylic acid cycle. Furthermore, within each group the radioactivity of carbon from each position was found to be the same within experimental error. If this was assumed to be true for the entire molecule, then comparison of the radioactivity from a particular position with that of the cholesterol as a whole indicated that 15 of the 27 carbons in cholesterol originate from the methyl group of acetate and 12 from the carboxyl group. This explains the 5 : 4 ratio mentioned above and

shows that the entire molecule of cholesterol can be built up from acetate as the sole source of carbon.

It was very desirable to identify these fifteen "methyl" and twelve "carboxyl" positions in the carbon skeleton of cholesterol, for the pattern of their distribution was likely to provide clues to the course of biosynthesis. In general, the first step was to prepare in quantity two samples of cholesterol: one biosynthesized from $^{14}CH_3CO_2H$ and the other from $CH_3{}^{14}CO_2H$. Then chemical methods were devised or adapted to break down the molecule in such a way that each of its carbon atoms could be isolated and assayed for radioactivity. After about six years of effort this task was completed in Bloch's laboratory in the United States of America and in ours. Bloch and his colleagues identified the origin of all the carbons in the side-chain (Würsch, Huang & Bloch, 1952) and three (C-18, C-13, C-7) of the ring carbons (Woodward & Bloch, 1953; Bloch, 1953), of which one (C-7) was independently isolated by Dauben & Takemura (1953) and the other two (C-18, C-13) by us; with our collaborators Hunter and Gore, we identified the other sixteen carbons (Cornforth, Hunter & Popják, 1953a, 1953b; Cornforth, Gore & Popják, 1957). The experience of the first degradation was confirmed throughout: the two groups of carbon atoms remained distinct, one group of fifteen atoms (marked m in formula III) originating from



(III)

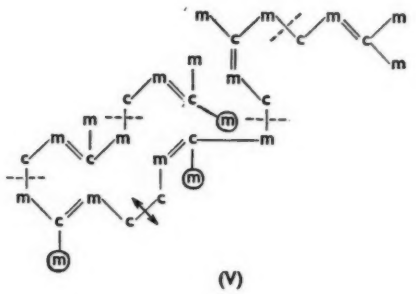
the methyl group of acetate and the other of twelve atoms (marked c) originating from the carboxyl group.

2. Squalene

It occurred to Bloch, when considering the pattern of the side-chain, that this could be correlated with an earlier hypothesis concerning the biogenesis of cholesterol. When Heilbron, Kamm & Owens (1926) were investigating the structure of squalene (IV),

(IV)

an unsaturated hydrocarbon found in large amounts in the liver of elasmobranch fishes, they suggested a possible biochemical relationship with sterols which was supported by the finding of Channon (1926) that squalene when fed to rabbits increased the cholesterol content of the liver. Robinson (1932) showed how the long molecule (IV) might be folded to give the sterol skeleton after loss of three methyl groups. Now squalene is a terpenoid hydrocarbon and conforms to the "isoprene rule": that is, it can be dissected into six "isopentane units", as shown in (IV). If each unit were built up from three molecules of acetic acid with loss of one carboxyl group, in a manner which had been postulated by Bonner & Arreguin (1949) for the biogenesis of another terpenoid (the rubber hydrocarbon), the distribution of m and c positions in the isopentane unit in squalene, and in cholesterol cyclized according to Robinson's scheme, would be as shown (V). The pattern in the side-chain obviously corresponds with that shown by experiment (III); the discrepancies evident in the ring structure had not then come to light.

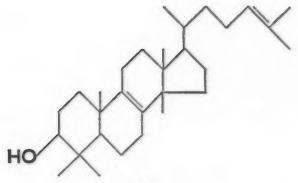


(V)

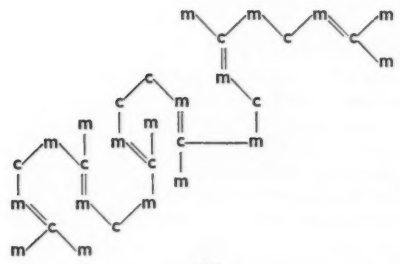
If squalene were an intermediate in the synthesis of cholesterol from acetate it must occur, however transiently, in the organs of mammals also. To demonstrate this, Langdon & Bloch (1953a) fed to rats radioactive acetate and large amounts of squalene; after some days the animals were killed and a little squalene was recovered from the livers. This squalene was now radioactive, indicating synthesis from acetate, and when it was fed to mice (Langdon & Bloch, 1953b) it was converted into cholesterol with an efficiency which seemed to be limited only by poor absorption. One of us (Popják, 1954 and unpublished work) confirmed these results and showed that other organs (e.g. the hen's ovary) which can synthesize cholesterol can also make squalene from acetate. We then prepared [^{14}C]squalene from [^{14}C]acetate and determined by chemical degradation the distribution of the two carbons of acetate in it (Cornforth & Popják, 1954). We found this to be identical with the pattern shown in (V).

3. Lanosterol

At about this time the structure of lanosterol, an alcohol found in wool-fat and (as "cryptosterol") in yeast, was established as that of a 4 : 4 : 14-trimethyl steroid (VI). Woodward & Bloch (1953) made the brilliant deduction that squalene might be cyclized to cholesterol via lanosterol. This requires a different method of folding and the migration of at least one methyl group (but see end of paragraph); the resulting pattern of m and c carbons in cholesterol is shown in (VII) and this is identical with the pattern (III) subsequently found by experiment. Evidence that lanosterol is indeed an intermediate in the biosynthesis was soon provided; Bloch and collaborators (Clayton & Bloch, 1956a, 1956b; Tchen & Bloch, 1957a) proved by ^{14}C-labelling that acetate and squalene are converted to lanosterol by rat-liver preparations



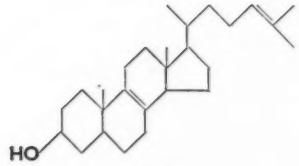
(VI)



(VII)

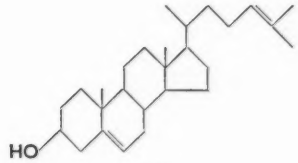
and that lanosterol is similarly converted to cholesterol. The synthesis of lanosterol in vivo from acetate in the liver of the normal rat has also been shown (Schneider, Clayton & Bloch 1957). When the cyclization of squalene to give lanosterol was conducted in water containing excess of deuterium or of ^{18}O, the lanosterol contained no excess of these isotopes; but when an excess of ^{18}O was present in the oxygen used during incubation, the hydroxyl group of lanosterol contained excess ^{18}O (Tchen & Bloch, 1957b). This suggests that squalene ⟶ lanosterol is essentially a one-step process, and the name "squalene oxidocyclase" has been suggested for the enzyme which effects it. It has been shown recently both in our laboratories and in Bloch's at Harvard that during cyclization of squalene to sterol a double shift of methyl groups occurs: the methyl group from position 14 migrates to position 13 and the methyl group from position 8 moves to position 14 (Cornforth, Cornforth, Pelter, Horning & Popják, 1958; Maudgal, Tchen & Bloch, 1958).

Between lanosterol and cholesterol, three methyl groups are lost and the double bond in the side-chain is reduced. Evidence has been obtained (Olson, Lindberg & Bloch, 1957) that all three methyl groups are eliminated as carbon dioxide; the 14-methyl group is apparently the first to go (Gautschi & Bloch, 1957), and is followed by the two at $C_{(4)}$, leaving zymosterol (VIII), another sterol of yeast which has also been



(VIII)

demonstrated to act as a precursor of cholesterol (Johnston & Bloch, 1957; Alexander & Schwenk, 1957a). The last intermediate is probably desmosterol (IX) (Stokes, Fish & Hickey, 1956), saturation of the 24 : 25-double bond finally giving cholesterol. Thus the details of those stages in the biosynthetic process which lie between squalene and cholesterol are being filled in rapidly; but the reactions leading from acetate to squalene have proved more elusive of identification.



(IX)

4. Earlier Stages of Biosynthesis

According to the original hypothesis of Bonner & Arreguin (1949) for the biogenesis of a five-carbon unit from which the rubber hydrocarbon could be built, two molecules of acetate form carbon dioxide and acetone; the acetone then reacts with a third molecule of acetate to give 3-methylcrotonate (X).

$$CH_3.CO_2H + CH_3.CO_2H \rightarrow CH_3.CO.CH_2 + CO_2$$
$$CH_3.CO.CH_3 + CH_3.CO_2H \rightarrow H_2O + (CH_3)_2.C{=}CH.CO_2H \qquad (X)$$

However, acetone does not seem able to act as an intermediate, at any rate in the biosynthesis of cholesterol, except in so far as it can be oxidized to acetate.

A somewhat different sequence, leading to the same end-product, can be postulated and has indeed been demonstrated in preparations of animal and plant cells. The successive stages, neglecting conjugation with coenzymes and possibly omitting some intermediates, are acetate, acetoacetate, 3-hydroxy-3-methylglutarate (XI), 3-hydroxy-3-methylbutyrate (3-hydroxy*iso*valerate) (XII) and 3-methylcrotonate (X).

$$CH_3.CO_2H + CH_3.CO_2H \rightleftharpoons CH_3.CO.CH_2.CO_2H + H_2O$$

$$CH_3.CO.CH_2.CO_2H + CH_3.CO_2H \rightleftharpoons CH_3.C(OH)(CH_2.CO_2H)(CH_2.CO_2H) \qquad (XI)$$

$$CH_3.C(OH)(CH_2.CO_2H)(CH_2.CO_2H) \rightleftharpoons CO_2 + CH_3.C(OH)(CH_2.CO_2H)(CH_3) \qquad (XII)$$

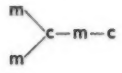
$$(XII)\quad CH_3.C(OH)(CH_2.CO_2H)(CH_3) \rightleftharpoons H_2O + (CH_3)_2.C{=}CH.CO_2H \qquad (X)$$

These reactions are formally reversible in that, for instance, 3-methylcrotonate can be degraded enzymically to acetate and acetoacetate, a molecule of carbon dioxide being taken up in the process (for review cf. Kennedy, 1957). In detail the picture is probably not so clear; thus Rudney & Ferguson (1957) recently showed that acetoacetyl-coenzyme A and acetyl-coenzyme A condense to give the mono-coenzyme A ester of 3-hydroxy-3-methylglutarate and a molecule of free

coenzyme A. This monoester is split enzymically to give free acetoacetate and acetyl-coenzyme A: both original reactants were therefore not regenerated.

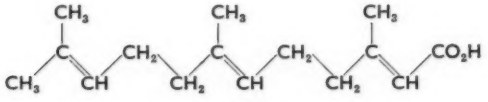
In vivo 3-methylcrotonate, or substances such as *iso*valerate (XIII) which can be considered to be in biochemical equilibrium with it, can function as a better source of carbon for cholesterol than can acetate (Bloch, Clark & Harary, 1954);

$$(CH_3)_2.CH.CH_2.CO_2H \qquad \text{(XIII)}$$



(XIV)

and this fact raised hopes that 3-methylcrotonate or a closely related substance was the postulated "isopentane" intermediate in squalene synthesis, especially as its enzymic synthesis from acetate gave the correct distribution (XIV) of m and c carbons (Rudney, 1955). However, the isotopic materials from which these high yields of cholesterol were obtained in vivo have always been labelled in the terminal isopropyl (or isopropylidene) group, so that fission to some form of acetoacetate in the manner outlined above might have preceded incorporation into cholesterol. In fact, the carboxyl carbon of *iso*valerate is a poor source of carbon for cholesterol (Zabin & Bloch, 1950); there is no evidence that (X), (XII) or (XIII) can be built into the cholesterol molecule as a five-carbon block; and although all four substances (X–XIII) furnish carbon for cholesterol when incubated with liver preparations, the yields have been disappointing in comparison with those from acetate. In an attempt to trace intermediates in a process by which squalene could be built up from one of these acids, a small amount of radioactivity from labelled 3-hydroxy-3-methylglutarate incubated with a particle-free liver preparation was found incorporated in an acid identified as farnesenic acid (XV),

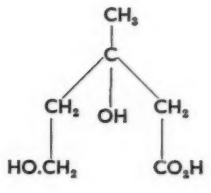


(XV)

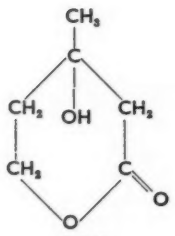
but the yields were very low (Dituri, Cobey, Warms & Gurin, 1956). The recent studies of Adamson & Greenberg (1957) make it unlikely that any of these branched chain acids is an obligatory intermediate in the synthesis of cholesterol.

5. Mevalonic Acid

In 1956 workers at the Merck Sharp & Dohme laboratories in New Jersey, USA, isolated from "distiller's solubles", a by-product of alcoholic fermentation, a substance promoting the growth of *Lactobacillus acidophilus* (Wright, Cresson, Skeggs, MacRae, Hoffman, Wolf & Folkers, 1956). This micro-organism normally will not grow in the absence of acetate; the new substance was able to replace acetate as a growth factor. The substance is now named mevalonic acid and it is an optically active form of 3 : 5-dihydroxy-3-methylpentanoic acid (XVI) (Wolf, Hoffman, Aldrich, Skeggs, Wright & Folkers, 1956).



(XVI)



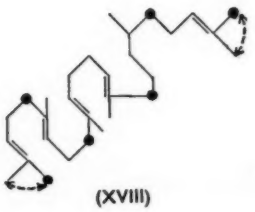
(XVII)

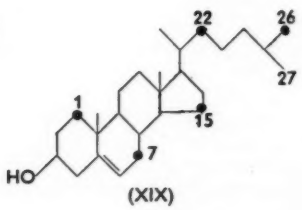
Its lactone (XVII) is easily hydrolysed; acid and lactone seem to be equivalent when used in biological systems. The close similarity of this substance to 3-hydroxy-3-methylglutaric acid (XI) prompted its discoverers to test it as an intermediate in the biosynthesis of cholesterol (Tavormina, Gibbs & Huff, 1956). When the synthetic, racemic lactone labelled with ^{14}C in the 2-position was added to a preparation of liver enzymes, over 40% of the total radioactivity could be recovered from cholesterol—an astonishingly efficient incorporation, especially if, as is probable, only one enantiomorph was used. When the experiment was repeated with mevalonate labelled in the carboxyl group (the 1-position), no radioactivity appeared in cholesterol but radioactive carbon dioxide was liberated (Tavormina & Gibbs, 1956). This observation suggested that an "isopentane" structure could arise from mevalonate by decarboxylation and that six of these units are built up to squalene which is then cyclized to sterol. We synthesized [2-^{14}C]mevalonate and found that enzyme preparations from rat liver, which in the presence of oxygen synthesized cholesterol from mevalonate, formed only squalene when oxygen was excluded (Popják, Gosselin & Gore, 1958; Popják, Gosselin, Gore & Gould, 1958). This was in harmony with the idea that the change from mevalonate to squalene is fairly direct, for the over-all process is a reduction:

$$6 \text{ mevalonate} + 2H \longrightarrow 1 \text{ squalene} + 6CO_2 + 12H_2O,$$

whereas the cyclization to lanosterol and the elimination of methyl groups are all oxidations, the only necessarily reductive stage after squalene being the saturation of the 24 : 25-double bond.

A larger amount of radioactive squalene was prepared from [2-^{14}C]mevalonate and degraded chemically (Cornforth, Cornforth, Popják & Gore, 1957, 1958). The squalene proved to be labelled at six positions only, and these positions (marked as black circles in formula XVIII) were those which were predicted by the hypothesis being tested: that mevalonate generates an "isopentane" unit, six of which are built into squalene. Dituri, Gurin & Rabinowitz (1957) have reported similar experiments, and recently Isler, Rüegg, Würsch, Gey & Pletscher (1957) have described a partial degradation of cholesterol biosynthesized from [2-^{14}C]mevalonate. The five


(XVIII)



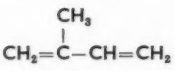
(XIX)

labelled positions predicted by theory are at C-1, C-7, C-15, C-22 and C-26 or C-27 (marked by black circles in formula XIX). Of these, C-7, C-22 and C-26 or C-27 were duly found to be radioactive, and the level of radioactivity indicated that a total of five labelled positions were present.

6. Present State of Knowledge

The discovery of mevalonic acid and of its significance as a precursor of cholesterol has divided the unknown sequence of reactions between acetate and squalene into two shorter sequences. How mevalonate is synthesized from acetate, and how squalene is synthesized from mevalonate, remain to be discovered. It is not yet certain whether the mevalonate molecule loses carbon dioxide before or after the association of units leading to squalene.

The suggestion that terpenoid compounds in general are built up from an "isopentane unit" such as isoprene (XX)



(XX)

was advanced some thirty years ago by Ruzicka. It accounts for the structure of many natural products and has been successful as a guide in elucidating unknown structures. The carotenoids, too, obey the "isoprene rule". The steroids can now be seen as re-arrangement products of squalene, a dihydrotriterpene; and Ruzicka's school have formulated plausible mechanisms whereby all the known types of triterpenoid structure could be derived from squalene also (Eschenmoser, Ruzicka, Jeger & Arigoni, 1955). To this collection of natural products must be added an increasing variety of substances, including many from moulds, in which one or more "isopentane units" seem to provide a part, but not all, of the carbon skeleton. Ergosterol (and perhaps the sitosterols) belong to this class, strictly speaking: the additional carbon atom in the side-chain (marked by black circle in formula II) does not originate from acetate, but can be supplied by formate (Danielsson & Bloch, 1957) or better by the S-methyl group of methionine (Alexander, Gold & Schwenk, 1957; Alexander & Schwenk, 1957b).

The possibility that mevalonate (which could theoretically give isoprene by loss of one molecule of carbon dioxide and two of water) is a key intermediate for all these natural products is being actively explored. Already evidence has been obtained that β-carotene is biosynthesized from mevalonate (Braithwaite & Goodwin, 1957) in a manner analogous to the synthesis of squalene.

We see from the history of these researches into the biogenesis of cholesterol that hypotheses, based on a comparative study of the molecular structure of natural products, have served as a guide to the solution of biochemical problems. In isotopes, now readily available, we have an unequalled tool for testing hypotheses and for demonstrating the biological conversion of one substance into another. Twenty years need no longer elapse between the formulation of a hypothesis on structural grounds and its critical testing in a biological system.

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THE ABSORPTION AND METABOLISM OF STEROLS

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2 Role of bile
3 Role of fat
4 Specificity of sterol absorption
5 Role of cholesterol esterase
6 Functions of intestinal mucosa in sterol absorption
  a Site of esterification of absorbed sterols
  b Permeability of the outer membrane of the mucosal cell
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8 Summary of evidence on sterol absorption
References

The biochemistry of cholesterol has in recent years been studied from many points of view, and the picture which is emerging is by no means a simple one. This paper is limited to a discussion of the absorption process and its regulation.

1. Introduction

Half a century ago, the absorption was first recognized when the addition of free or esterified cholesterol to the food of experimental animals raised their blood cholesterol levels (Beneke, 1880; Pribram, 1906; Dorée & Gardner, 1909; Fraser & Gardner, 1909). Later work indicated that many aspects of the process were intimately connected with the absorption of fat. More recently, however, work in many laboratories has shown the earlier concepts to be inadequate, and a new working hypothesis has been advanced (Glover & Green, 1956).

The lipolytic hypothesis (Pflüger, 1900; Bloor, 1922; Verzár & McDougall, 1936) applied to neutral fat postulated hydrolysis in the lumen of the intestine, the products being passed through the mucosal membrane and recombined before entering the lymph capillaries. Free (or esterified) cholesterol given by mouth to the dog appeared in the chyle as ester (Mueller, 1915, 1916). This led to the extension of the lipolytic hypothesis to the absorption of cholesterol. A cholesterol esterase was later shown to be present in the gut wall (Frölicher & Süllmann, 1934) and, like pancreatic esterase, it exhibited both hydrolytic (Klein, 1939) and synthetic activity (Schramm & Wolff, 1940; Nieft & Deuel, 1949; Swell, Byron & Treadwell, 1950; Swell, Boiter, Field & Treadwell, 1955). These ideas provided a simple framework for an extended picture.

2. Role of Bile

An early extension was to bring in the effect of bile. Addition of bile salts (0.5%) to a cholesterol-supplemented diet improved the absorption of the sterol by the rabbit (Schönheimer, 1924; Member, Bruger & Oppenheim, 1944) and by the rat (Alfin-Slater, Schotz, Greenberg & Deuel, 1952). That the increased sterol contents of blood and tissues reflected greater absorption was confirmed by measuring sterol concentrations in the lymph (Kim & Ivy, 1952; Siperstein, Chaikoff & Reinhardt, 1952). Cholate is more effective than deoxycholate (Swell, Field & Treadwell, 1953).

It seems clear that bile is needed if absorption is to occur at all freely (Bloom, Chaikoff, Reinhardt, Entenman & Dauben, 1950; Friedman, 1956), but the question remains open whether without bile there can be any absorption at all. Very small amounts of cholesterol may possibly be absorbed in the absence of bile (Friedman, Byers & Shibata, 1953) if dispersion is effected by some other agency.

The well-known role of bile salts in forming soluble complexes (Wieland & Sorge, 1916) may also, in the case of cholesterol, improve the accessibility of the substrate to the action of cholesterol esterase. Thus, the hydrolysis of esters is greatly increased in their presence (Klein, 1939). Again, the bile salts appear to be essential for the synthetic action of the enzyme (Swell *et al.* 1953).

3. Role of Fat

Dietary fat has been shown to improve the absorption of cholesterol in many animal species and thus leads to a de-

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BIOSYNTHESIS OF CHOLESTEROL

Continued from page **225**

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creased sterol excretion (Cook, 1936; Kim & Ivy, 1952; Pihl, 1955) and an increased blood cholesterol level (taken here as an indirect measure of enhanced absorption) (Swell, Flick, Field & Treadwell, 1954).

Free fatty acids exert a greater effect than neutral fat, provided that they are unsaturated; palmitic and stearic acids tend to inhibit cholesterol absorption (Kim & Ivy, 1952) and may cause a threefold increase in faecal excretion of cholesterol under conditions such that oleic and linoleic acids make no change (Lin, Karvinen & Ivy, 1956). Other workers (Swell, Flick, Field & Treadwell, 1955) confirm that unsaturated acids favour an early rise in blood cholesterol. As compared with saturated acids, the low-melting unsaturated acids may be more easily dispersed in the aqueous phase at body-temperature and they are probably used preferentially in the esterification of cholesterol by the enzyme (Swell, Flick, Field & Treadwell, 1955; Hernandez & Chaikoff, 1957).

Dietary fat thus (i) acts as a vehicle for the sterol; (ii) aids its dispersion in the lumen of the gut by virtue of the detergent action of enzymically formed mono- and di-glycerides; (iii) stimulates bile flow with resultant actions of bile salts; and (iv) provides a supply of fatty acids for esterifying cholesterol (Swell, Flick, Field & Treadwell, 1955).

It does not seem to follow that cholesterol cannot be absorbed on a fat-free diet, at any rate by the rabbit (Dubach & Hill, 1946; Popják, 1946), or rat (Bollman & Flock, 1951; Swell, Flick, Field & Treadwell, 1955; Pihl, 1955).

The similarity in behaviour of cholesterol and neutral fat during intestinal absorption made it reasonable to consider that the two were absorbed together (Frazer, 1955) and that one function of fat was to carry the sterol in solution across the mucosal membrane. Evidence based partly on isotope studies shows that incompletely hydrolysed neutral fat (Reiser, 1954; Frazer, 1955) and phospholipid molecules (Blomstrand, 1954) can penetrate the mucosal membrane. Nevertheless transport by simple solution is too uncomplicated a concept, since it does not account for the marked selectivity of the intestine in absorbing some sterols and carotenoids and rejecting others.

4. Specificity of Sterol Absorption

Early work on the composition of faecal lipids following ingestion of various sterols by animals or men revealed that the saturated sterols—coprosterol (Bondzýnski, 1896; Müller, 1900), cholestanol (Boehm, 1911)—and also the phytosterols were poorly absorbed in comparison with cholesterol (Schönheimer, 1931).

Later work (summarized in Table I) with labelled compounds gave quantitative confirmation. It will be seen from fig. 1 that, as between the poorly absorbed phytosterols and cholesterol or 7-dehydrocholesterol—which are well absorbed—the differences in structure are indeed small. These differences moreover are not accompanied by appreciable differences in fat solubility.

Inhibition of Cholesterol Absorption by Other Sterols

Another facet of the problem is that the poorly absorbed sterols tend to inhibit the absorption of cholesterol. Thus Horlick & Katz (1949) observed that addition of 1% cholesterol to the diet of chickens produced a sixfold increase in the blood cholesterol level after one week, followed by a more

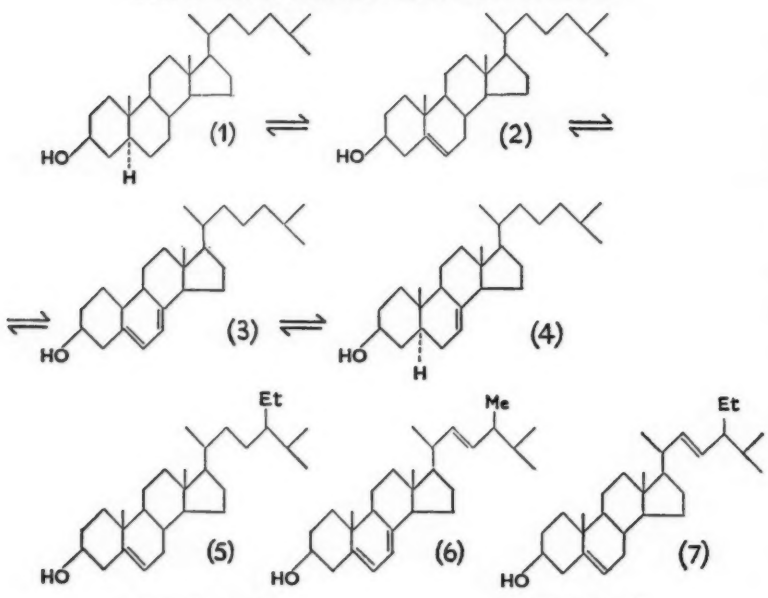
TABLE I. ABSORPTION OF STEROLS BY VARIOUS ANIMAL SPECIES

Animal species	Sterol	Absorption of dose (%)	Reference
Rat	Cholesterol	30–50	Cook & Thomson (1951), Chaikoff, Bloom, Siperstein, Kiyasu, Reinhardt, Dauben & Eastham (1952), Cook (1952), Favarger & Metzger (1952), Friedman, Byers & Shibata (1953)
	[^{2}H]Cholestanol	27	Roth & Favarger (1955)
	[^{3}H]Cholestanol [^{14}C]Cholestanol	10	Gould, Lotz & Lilly (1956)
	[^{14}C]Ergosterol	2–5	Hanahan & Wakil (1953)
	[^{2}H]β-Sitosterol	28	Roth & Favarger (1955)
	[^{3}H]β-Sitosterol	8	Gould *et al.* (1956)
Rabbit	Cholesterol	80–90	Cook & Thomson (1951), Cook, Kliman & Fieser (1954)
	Cholestanol	54–86	Cook *et al.* (1954)
	Lathosterol	80	Cook *et al.* (1954)
	7-Dehydrocholesterol	90	Cook *et al.* (1954)
Guinea-pig	Cholesterol	46–50	Cook & Thomson (1951)
	[^{14}C]Ergosterol	1–5	Glover, Leat & Morton (1957)
	[^{14}C]Leaf-sterols (sitosterol)	5	Duncan (1954)
Dog	Cholesterol	50–80	Favarger & Metzger (1952), Mosbach & Abell (1954)
Man	Cholesterol	57–75	Favarger & Metzger (1952)
	Cholesterol (in eggs)	60	Cook, Edwards & Riddell (1956)
	Cholesterol (crystalline in oil)	15	
	[^{3}H]Sitosterol	>4	Gould *et al.* (1956)

gradual rise to a plateau, and that atheroma developed within a few weeks. Soon afterwards Peterson (1951) found that the further addition of 1% soya-bean sterols (mainly sitosterol) to the above atherogenic diet prevented in the chickens (i) the marked increase in cholesterol contents of plasma and liver and (ii) the occurrence of atherosclerosis. This work reinforced the earlier experience of Sperry & Bergmann (1937) who found that administration of sitosterol to mice lowered the liver cholesterol content. The authors regarded the sitosterol as interfering with the reabsorption of endogenous cholesterol secreted into the intestine. This effect, which is obtained only with free sterols (Peterson, Schneour, Peek & Gaffey, 1953) has been confirmed in the rabbit (Pollak, 1953a), rat (Hernandez, Peterson, Chaikoff & Dauben, 1953) and man (Pollak, 1953b; Best, Duncan, Van Loon & Wathen, 1954; Barber & Grant, 1955; Farquhar, Smith & Dempsey, 1956). The addition of cholestanol (2–5%) to the diet also inhibits the absorption of cholesterol in the chicken (Siperstein, Nichols & Chaikoff, 1953), rabbit (Nichols, Siperstein & Chaikoff, 1953), and rat (Rosenman, Byers & Friedman, 1954; Daskalakis & Chaikoff, 1955).

The interference is greatest when the dietary inhibiting sterols preponderate over cholesterol by a factor of 2–3 (Peterson *et al.* 1953). Whereas all reports agree that cholestanol reduces cholesterol absorption, experience with sitosterol has varied, particularly in experiments using single doses

FIG. 1. SOME ANIMAL AND PLANT STEROLS



Animal sterols

1. Cholestanol
2. Cholesterol
3. 7-Dehydrocholesterol
4. Lathosterol

Plant sterols

5. β-Sitosterol
6. Ergosterol
7. Stigmasterol

of the "inhibitor". Hernandez *et al.* (1953) and Hernandez & Chaikoff (1954) found that soya-sterols and sitosterol respectively caused a marked reduction in the absorption of [4-^{14}C]-cholesterol into the lymph of cannulated rats. In a somewhat similar experiment, however, Rosenman, Byers & Friedman (1954) and Friedman, Rosenman & Byers (1956), studying the capacity of sitosterol at different levels to inhibit cholesterol absorption in the rat, observed only a slight effect at most. Again, although groups of workers have noted a 10–20% decline in the cholesterol level of hypercholesterolaemic patients given 5–25 g. "sitosterol" per day over a period of at least several weeks, Wilkinson, Boyle, Jackson & Benjamin (1955) were unable to observe any significant change in their patients similarly treated. It may be that sitosterol is less effective than cholestanol. From Table I it can be seen that the latter is itself absorbed to a greater extent than the former. Moreover, administered cholestanol tends to accumulate in the tissues of the animal; and in the rat, after feeding it for only 5 days, approximately 25% of the tissue sterol had been replaced by the cholestanol, whereas under similar conditions retained sitosterol made up only 2–3% of the total sterols (Gould, Lotz & Lilly, 1956). The capacity of the intestine to absorb cholesterol may depend on a rapid exchange and transport of cholesterol between the mucosal lipoproteins (Glover & Green, 1956; and see below). On this basis foreign sterol present in the mucosa may immobilize the specific lipoproteins and so reduce the capacity of the system to transport cholesterol. For example β-sitosterol and ergosterol, because they are poorly absorbed, will be less efficient inhibitors than cholestanol, particularly in experiments involving only single doses (as found by Daskalakis & Chaikoff, 1955).

5. Role of Cholesterol Esterase

The extent to which cholesterol absorption is impaired appears to depend on the relative proportions of phytosterol and cholesterol in the diet. This suggests that the inhibition is competitive and implies the participation of a protein. Such a protein might well be an enzyme such as cholesterol esterase, and this idea has led to detailed studies in vivo and in vitro.

i. *In Vivo.* As the lymphatic route is the only way by which dietary sterol molecules enter the body intact (Biggs, Friedman & Byers, 1951; Chaikoff, Bloom, Siperstein, Kiyasu, Reinhardt, Dauben & Eastham, 1952), studies were directed to intestinal lymph.

The cholesterol concentration in such lymph from the fasting animal is about one-third that in plasma. The sterol is present in both free and esterified forms and is associated with the same lipoproteins in both lymph and plasma. The two main ones exhibit in the ultracentrifuge $-S_{1.21}$ values[1] in the range 4–7 and at 23 respectively. A small amount of a third fraction (chylomicra; $-S_{1.21} > 70$) is also present (Page, Lewis & Plahl, 1953). During the absorption of fat there is a slight increase in the sterol content of lymph (Brockett, Spiers & Himwich, 1934; Page *et al.* 1953), mainly in the fraction having $-S_{1.21} > 70$, but there is no apparent change in the others (Byers & Friedman, 1954; Albrink, Clenn, Peters & Man, 1955).

Of the total sterol in the lymph, 70% is normally esterified, and, during the absorption of [4-^{14}C]cholesterol, radioactivity appears in both the ester and free fractions. This supports the earlier observations of Mueller and others that esterase participates in absorption. However, using [4-^{14}C]cholesterol Hellman, Rosenfeld, Gallagher, Adlersberg & Wang (1953) detected the labelled sterol in the lymph earlier in the free than in the esterified fraction, indicating that esterification may not be obligatory. Again, Hernandez, Chaikoff, Dauben & Abraham (1954) demonstrated that *epi*cholesterol, which is not esterified by cholesterol esterase, was absorbed into the lymph, although only at half the rate characteristic of cholesterol. This confirms that esterification is not indispensable, although it probably speeds up absorption.

The work of Daskalakis & Chaikoff (1955) points away from the idea that cholesterol esterase is the selective agent in absorption. Thus it was shown for rats that, although cholestanol and ergosterol mixed with the same dose of [4-^{14}C]cholesterol depressed the absorption of the latter into the intestinal lymph by 80% and 50% respectively, the percentage of [4-^{14}C]cholesterol in the esterified form did not vary.

ii. *In Vitro.* Cholesterol esterase was found to be not highly specific. Cholestanol is more readily esterified than cholesterol, and β-sitosterol very slightly less so (Swell, Field & Treadwell, 1954; Hernandez & Chaikoff, 1957), yet both are inhibitors of cholesterol absorption.

[1] Negative sedimentation or flotation in a medium of density 1.21.

6. Functions of Intestinal Mucosa in Sterol Absorption

a. Site of Esterification of Absorbed Sterols

Investigations on the locus of esterification in the gut wall showed cholesterol esterase to be widely distributed in the intestine. Esterification might then take place in the mucosal cell or in the underlying connective tissue or in the lymphatic capillaries.

Using the fasting guinea-pig, examination of the lipids from intestinal mucosa either by the digitonide method (Glover, Glover & Morton, 1952) or by a paper chromatographic procedure (Glover & Green, 1956) showed that sterol esters were absent. This was confirmed for the fasting rat (Stainer, 1957). Again, in the same species, after single doses of 7-dehydrocholesterol or cholesterol (150–200 mg. in arachis oil), only very occasionally is a trace of ester detectable in the mucosa. However, when guinea-pigs are maintained on a 1% cholesterol diet for a week, some 25–30% of the mucosal cholesterol is esterified (Green, 1955). Similarly, Favarger & Metzger (1952) could not detect esterification of [^{2}H]cholesterol in the mucosa of rats given single doses. Under these conditions the connective tissue between the mucosal cells and the lymphatic capillaries is the most likely site, since the amount of ester within the lymph is steady in the range 65–75%. The presence of esters in the mucosa after seven days on a 1% cholesterol diet results from an active uptake of the cholesterol from the lumen, faster than its rate of removal at the rear of the cell. Esterified cholesterol disappears when the intake declines. An explanation of this is given later (section 6c).

The role of cholesterol esterase thus includes: (i) hydrolysis of esters in the lumen before absorption; (ii) formation of innocuous temporary stores during periods of excessive absorption; and (iii) increasing the rate of removal of cholesterol from mucosa and connective tissue into lymph.

b. Permeability of the Outer Membrane of the Mucosal Cell

It thus appears that the block in phytosterol absorption may occur before esterification, and the cell membrane may be the main barrier. Repeated observations have shown that phytosterols administered in oil are largely excreted. This indicates that they become separated from the fat and are unable to penetrate the mucosa. Recent experiments by Roth & Favarger (1955) showed that the percentage absorption of [^{2}H]tripalmitin administered to rats alone or in the presence of [^{2}H]cholesterol, [^{2}H]sitosterol or [^{2}H]ergosterol was constant at about 67% but the absorptions for the sterols were respectively 47%, 28% and 27%. To quote the authors: "La paroi intestinale semble traiter chaque lipide individuellement, comme si elle triait les molécules une à une."

The above studies and others noted in Table I, in which [^{14}C]ergosterol and [^{3}H]sitosterol were administered to guinea-pigs and rats, showed conclusively that out of the small amounts of phytosterol which were absorbed only a mere fraction was present as intact sterol in the gut wall. Again the initial block appears to be the transport of the sterol from the lumen into the mucosal cell.

c. Distribution of 7-Dehydrocholesterol in Mucosal Cells

Parallel work arising out of a study on the distribution of provitamin D_3 (7-dehydrocholesterol) in the various tissues of experimental animals (Glover *et al.* 1952) led to the same conclusion. Examination of sterols from the intestinal mucosa of the fasting guinea-pig showed the proportion of 7-dehydrocholesterol to be constant (Glover & Green, 1954). In animals absorbing cholesterol or 7-dehydrocholesterol, the proportion changed not only through the presence of the absorbed sterol per se but also because of the action of a sterol dehydrogenase (or reductase) system later shown to catalyse the reduction of 7-dehydrocholesterol to cholesterol (Glover *et al.* 1952) and lathosterol (Glover & Green, 1956). The reduction of dietary 7-dehydrocholesterol to lathosterol has also been encountered in the rat (Glover & Stainer, 1956). Fractionation of the mucosal cells by the Schneider-Hogeboom technique (Schneider & Hogeboom, 1950) was then carried out to ascertain the distribution of 7-dehydrocholesterol among the organelles. It was also possible to tell if sterol undergoing absorption at the same time as neutral fat was contained in the fatty globule fraction (as might have been expected from the modified particulate absorption theory of Frazer (1953, 1955)).[2] The results (Glover & Green, 1954, 1957) display the pattern of distribution in fasting guinea-pigs and in animals killed six hours after a dose of cholesterol or 7-dehydrocholesterol (150–200 mg. in 1 ml. arachis oil) as shown in Table II. Similar results (Table III) have been obtained in experiments on the absorption of 7-dehydrocholesterol by the rat (Glover & Stainer, 1956).

TABLE II. DISTRIBUTION OF 7-DEHYDROCHOLESTEROL IN THE MUCOSA OF THE GUINEA-PIG DOSED WITH CHOLESTEROL AND 7-DEHYDROCHOLESTEROL

Cell fraction	Controls (9 experiments)	Animals dosed with: 7-dehydrocholesterol (3 experiments)	Animals dosed with: cholesterol (3 experiments)	Animals dosed with: [^{14}C]cholesterol (2 experiments)
	Percentage of total sterol ± S.D.			Specific activity of total sterol (counts/min./mg.)
Homogenate	9.2 ± 0.5	14.4 ± 0.1	6.9 ± 0.4	— —
Cell membrane + nuclei	9.7 ± 1.3	15.5 ± 2.9	6.7 ± 1.2	63, 14
Mitochondria	8.8 ± 1.8	15.4 ± 4.5	6.5 ± 1.1	71, 15
Microsomes	9.9 ± 1.3	16.9 ± 3.1	7.3 ± 0.5	68, 12
Groundplasm	8.2 ± 1.3	12.7 ± 1.0	6.7 ± 3.3	60, 13
Fatty layer	—	—	—	59 —

TABLE III. DISTRIBUTION OF 7-DEHYDROCHOLESTEROL AND LATHOSTEROL IN THE MUCOSA OF THE RAT DOSED WITH 7-DEHYDROCHOLESTEROL (results expressed as a percentage of total sterols)

Cell fraction	Controls (9 experiments)		Dosed (8 experiments)	
	Δ5:7-stenol	Δ7-stenol	Δ5:7-stenol	Δ7-stenol
Homogenate	2.7	11.4	20.4	50.0
Cell membrane + nuclei	2.6	8.9	18.9	55.6
Mitochondria	3.6	10.9	18.5	58.8
Microsomes	1.9	9.5	21.7	53.6
Groundplasm	2.8	8.9	15.2	45.5
Fatty layer	2.2	11.1	14.5	45.5

[2] See paper by Frazer in this number of the Bulletin, p. 212.—Ed.

The different sterols thus exist in the same relative proportions in the various cell components. Further, the administered sterol on absorption quickly acquires a similar distribution. In addition, lathosterol—newly formed from 7-dehydrocholesterol—is also spread over the cell components (Table III).

After obtaining the various cell fractions, mitochondria, microsomes and groundplasm, from animals dosed with [^{14}C]cholesterol, it was found (Green, 1955) by electrophoresis on paper that the labelled sterol was present in lipoproteins.

Exchange of sterols between the different cell fractions via lipoproteins is postulated and must be more rapid than the enzymic reduction of 7-dehydrocholesterol because the resulting lathosterol is also evenly dispersed. Hence the dietary sterol must be disaggregated to molecules for complete mixing with endogenous sterol. These experiments imply moreover that the sterols undergoing absorption are transported across the mucosa by making and breaking their attachment to the lipoproteins and not by the migration of lipid droplets. Exchange of this kind between the lipoproteins of plasma and blood cells has already been observed by Gould (1951) and by Hagerman & Gould (1951). More recently, exchange between free cholesterol in the liver and that in the plasma has been noted (Gould, 1955).

Glover & Green (1956) found that exchange between the fractions and artificially dispersed sterols does not occur at 0° C.; metabolism thus seems to be necessary for the uptake of sterols by the lipoproteins. Again, the fact that cholesterol esters can accumulate in mucosal cells during absorption against a concentration gradient also implies that the process is dependent on metabolic energy. The nature of the forces binding the sterol in the cell various fractions is far from clear.

By comparing the concentration of total sterol in relation to the nitrogen content in each of the cell fractions (Table IV), it has been possible to show that each fraction has the capacity to take up absorbed sterols additional to those normally present in the fasting state.

TABLE IV. UPTAKE OF DIETARY STEROLS BY THE ORGANELLES OF INTESTINAL MUCOSA

Cell fraction	Total sterol (mg./mg.N)			
	Guinea-pig		Rat	
	Controls (3 experiments)	Dosed (3 experiments)	Controls (4 experiments)	Dosed (8 experiments)
Cell membrane + nuclei	0.13 (0.11—0.15)	0.39, 0.30*	0.21±0.02 (0.19—0.23)	0.52±0.25 (0.26—0.94)
Mitochondria	0.19 (0.18—0.21)	0.23 (0.21—0.27)	0.23±0.05 (0.18—0.28)	0.45±0.14 (0.31—0.67)
Microsomes	0.31 (0.28—0.33)	0.41 (0.34—0.45)	0.36±0.03 (0.34—0.40)	0.52±0.07 (0.42—0.62)
Groundplasm	0.03 (0.02—0.03)	0.11, 0.07*	0.05±0.02 (0.03—0.05)	0.08±0.03 (0.06—0.15)

Figures in parentheses represent range of values found.

* Two determinations only

The lipoprotein transport system for free cholesterol may, however, be limited in capacity, and excess cholesterol entering the cell will be esterified to form a temporary reserve lasting until the peak absorption period is over. The absence of esterified cholesterol in the mucosal cells of the fasting or singly dosed animal, although the esterase is known to be present, is particularly noteworthy. Now Metzger & Favarger (1952) have shown that cholesterol bound to the lipoproteins in plasma does not become esterified with oleic acid under the action of an intestinal mucosal homogenate which readily esterifies free cholesterol dispersed in an artificial medium. It seems likely then that the excess sterol entering the mucosal cell, which cannot be held by the lipoprotein through the OH-group, is rendered innocuous by cholesterol esterase.

7. Sterols in the Intestinal Lumen

a. Excretion

Not all the sterol absorbed from the lumen of the intestine is passed into the lymph; some of it will be excreted. It has long been known (Sperry & Angevine, 1932) that secretion and partial reabsorption of lipids can occur across the mucosal membrane. Schönheimer & von Behring (1930) and Schönheimer & Hrdina (1932) found that the cholestanol of faeces was derived from cholesterol; and two isomeric sterols—lathosterol (Δ^7-cholestenol) and Δ^7-coprostenol—have been detected recently by Baumann and co-workers (Wells, Coleman & Baumann, 1955; Coleman & Baumann, 1957a; Coleman & Baumann, 1957b). The former comes from 7-dehydrocholesterol by enzyme action in the mucosa and the latter is the result of bacterial reduction in the colon. The bacterial reductions have also been confirmed in vitro (Snog-Hjaer, Prange & Dam, 1956; Coleman & Baumann, 1957b) and in vivo (Rosenfeld, Hellman & Gallagher, 1956). Wells *et al.* (1955) found lathosterol to constitute 40% of faecal sterols in the rat, whereas those of intestinal mucosa contain only 10% (Moore & Baumann, 1952; Glover & Stainer, 1956). Similarly, the lipid dispersed in the fluid secreted into the lumen of the small intestine of the fasting rat yields on extraction a sterol mixture containing more lathosterol than cholesterol (Stainer, 1957). Since both these sterols can cross the mucosal cell membrane, it would seem that the latter is reabsorbed preferentially. Cook, Kliman & Fieser (1954) found lathosterol to be slightly less well absorbed than cholesterol.

b. Form of Sterol in the Lumen

Complete mixing of dietary and endogenous sterols within the mucosal cells implies dispersion of the former to individual molecules—possibly before there is traffic across the cell membrane. To check this the intestinal fluids from the fasting rat and the dosed rat have been investigated (Stainer, 1957). Mucoproteins were precipitated (in the high-speed centrifuge) following the removal of food debris, and they were shown to carry some of the dietary sterol. Portions of the precipitate were subjected to paper electrophoresis in borate buffer (pH 10.5). Many zones were detected by standard procedures. Two zones were stained pink with antimony trichloride, thus indicating the presence of sterol, and these corresponded with two of four protein zones that stained with naphthalene black. This evidence, coupled with the fact that the sterols in these fractions are not extractable with ether unless the protein is first denatured, puts greater emphasis on the mucoprotein fraction as a hydrotropic carrier of sterol and

indicates a very close binding between sterol and mucoprotein molecules in the lumen. Bile acids were not detected in the fraction, so presumably the mucoproteins, as originally suggested by Favarger & Roth (1954), are the hydrotropic carriers required for the absorption and the secretion of sterols, the net effect depending on the presence or absence of cholesterol in the diet.

As this mucous secretion passes down the intestine it probably becomes digested, liberating the sterols and other lipids carried. The sterols liberated in the proximal region may be taken up in the distal region by fresh mucoprotein. During its lifetime, however, the mucoprotein carrier will have the capacity to exchange secreted sterol such as cholestanol (which is not readily absorbed) for dietary cholesterol and enable the latter to enter the mucosal cells.

c. Effect of Dietary Protein

If the above view is sound, then an increase of mucoprotein in the lumen might enhance absorption of either dietary sterol or endogenous sterol, e.g. from bile. Dietary protein could perhaps exert a sparing action on the mucoprotein by delaying its digestion, and by providing the amino acids within the mucosa it could increase the rate of its biosynthesis. Many workers report that a high-protein diet (protein $>15\%$ of food) raises blood cholesterol levels and increases the chance of atherosclerosis in adult animals (Newburgh & Clarkson, 1923; Meeker & Kesten, 1941; Jones & Huffman, 1956). High-protein intakes have no marked effect, however, on the blood cholesterol level of young growing animals (Okey & Lyman, 1956). Other evidence supports these findings: thus Page *et al.* (1953) found that administration of casein to rats caused a quick rise (i.e., within two hours) in the sterol of intestinal lymph. Again, β-carotene, which becomes associated with the mucoprotein fraction (Glover, 1956, unpublished observations), is utilized better by rats on a high- than on a low-protein diet (Arnrich & Pederson, 1956). Finally, cholesterol ingested in the form of eggs, where it is present as a lipoprotein, is more efficiently and rapidly absorbed than cholesterol fed with olive-oil (Okey & Stewart, 1933; Messinger, Porosowska & Steele, 1950; Cook, Edwards & Riddell, 1956).

8. Summary of Evidence on Sterol Absorption

The hypothetical scheme shown in fig. 2 reduces to order most of the evidence, if the following statements are taken in conjunction:

i. Proteolytic enzymes and cholesterol esterase ensure eventually that all dietary sterol becomes free. Bile salts aid the dispersion of all lipids, including cholesterol, and stimulate the esterase.

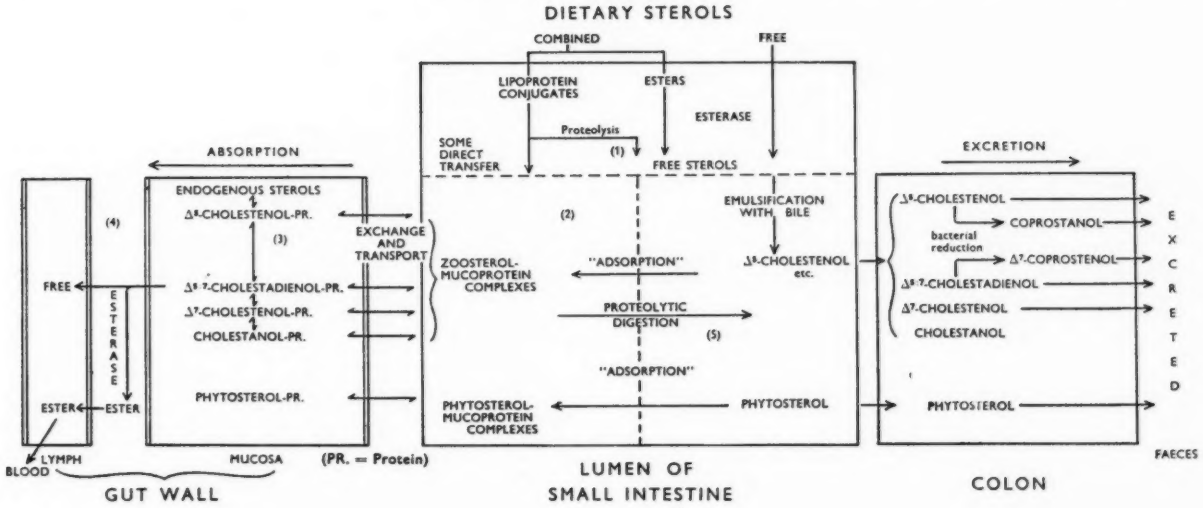
ii. The colloidal lipid particles and micelles are trapped in the brush border of the villi where contact with the gelatinous mucoprotein is established and sterol exchange can take place. Some direct transfer of sterol from ingested lipoproteins may also occur. The transfer is regarded as taking place more quickly with cholesterol than with a phytosterol.

iii. Exchange of the sterols then occurs between the mucoprotein complex and the cell membrane, and transport within the cell is effected as a result of successive transfers to and from other lipoproteins. Acceptance of individual sterols by proteins shows high structural specificity; this cannot be accounted for in terms of simple adsorption. Sol–gel transformations may play a part in the make and break of the link.

The poor absorption of phytosterols is attributed to their slow rate of reaction with the animal type of lipoprotein compared with cholesterol; the latter is, moreover, present in the cell in excess. Each successive change for a phytosterol molecule becomes more difficult the more deeply it penetrates the cell where the lipoproteins are already loaded with cholesterol.

However, when a phytosterol molecule succeeds in attaching itself, that transfer centre is probably immobilized for a period which would allow several transfers of a cholesterol molecule. The observation that cholestanol, which is absorbed more readily than ergosterol, is also a more effective inhibitor of

FIG. 2. DIAGRAM ILLUSTRATING THE MAIN FEATURES OF THE INTESTINAL ABSORPTION AND EXCRETION OF STEROLS



cholesterol absorption (Daskalakis & Chaikoff, 1955) supports this concept.

iv. The sterols probably leave the mucosal cells by a similar process. The fluid in the intercellular spaces contains lipoproteins which may take part in this exchange, but, when there is more than enough free sterol to saturate these lipoproteins, the liberated excess will become esterified in the presence of fatty acids. Thus a small proportion of free sterol and a large proportion of ester will enter the lymph capillaries and eventually pass to the blood stream. Esterification will also occur within the mucosa during the prolonged administration of cholesterol when the transport system becomes overloaded.

v. The concentration of mucoprotein "carrier" in the lumen of the intestine at any time is determined by the difference between rates of elaboration and secretion by the mucosa and of destruction by enzymic breakdown. The magnitude of the surplus probably influences the efficiency of cholesterol absorption.

vi. The sterols liberated from the mucoprotein–sterol complexes, together with unabsorbed dietary sterols, eventually reach the colon, where bacterial reduction of the 5 : 6-double bond (in cholesterol, β-sitosterol and 7-dehydrocholesterol) takes place. Acidic and ketonic degradation products appearing in the faeces (Riddell & Cook, 1955; Edwards & Cook, 1955) may also in part be formed in the colon.

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REMOVAL OF LIPIDS FROM THE BLOOD STREAM

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1 Removal of lipoproteins from the blood
  a Permeability of the capillary membrane to lipoproteins
  b Direct uptake of lipoprotein lipid from the blood
2 Removal of chylomicron lipid from the blood
  a Removal of chylomicron lipid as particles
  b Degradation of chylomicrons in the blood: the heparin-clearing reaction
3 Removal of unesterified fatty acids from the blood
References

The lipids are for the most part insoluble in water, and in the blood they do not occur in the free state but are associated with each other and with proteins as complex water-soluble molecules (lipoproteins) and as emulsion particles (chylomicrons). In the post-absorptive state (a term used to define a state when neither absorption from the gut nor mobilization from the fat depots is occurring), the lipids in the plasma—fatty acids, triglycerides, phospholipids and cholesterol and its esters—are found in one or other of the two classes of lipoprotein complexes known as the α- and β-lipoproteins (Oncley, 1956). These are maintained at a more or less steady concentration by processes which occur predominantly in the liver. When absorption of a fatty meal occurs, additional lipid, almost entirely in the form of chylomicrons, enters the blood stream from the thoracic duct and causes a transient increase in the concentration of total lipid (Bergström & Borgström, 1955). The chylomicrons consist predominantly of triglyceride, but they also contain a small proportion of cholesterol and phospholipid, and in the blood they are apparently stabilized by a surface layer of protein (Frazer, 1949; Robinson, 1955). The form in which fat is mobilized from the depots is less well defined; it may be partly as triglyceride in lipoproteins and chylomicrons or as unesterified fatty acid. The latter occurs in the blood as a soluble complex with the plasma albumin (Shapiro, 1957).

In general, the removal of lipids from the blood is determined by the permeability of the blood capillary membrane to the various lipid complexes or to their lipid components. However, at certain sites, cells in contact with the blood, such as reticulo-endothelial cells or possibly hepatic parenchymal cells, may be able to take up lipids directly.

1. Removal of Lipoproteins from the Blood

The lipid components of the lipoproteins are apparently removed from the blood continuously at a rate which balances their entry; a steady concentration is thus maintained in the plasma. When homologous lipoproteins, containing isotopically labelled cholesterol or phospholipid, are introduced into the blood stream in a way which does not alter their total concentration, the lipid constituents equilibrate rapidly with the lipids of the extravascular tissues. The fall in the concentration of the isotope in the blood during this period of equilibration indicates the rate at which the lipids are removed from the circulation. Under such conditions the circulating half-life of labelled plasma phospholipid has been found to be 1 hour or less in rats (Zilversmit & Bollman, 1956), 1½ hours in rabbits (Hahn & Hevesy, 1939) and 5–8 hours in dogs (Zilversmit, Entenman, Fishler & Chaikoff, 1943; Zilversmit, Entenman & Chaikoff, 1948). Similar values have been obtained for the rate of removal of cholesterol in dogs following the transfusion of lipoproteins labelled in the cholesterol moiety (Gould, 1951). Most of the cholesterol and phospholipid from the plasma lipoproteins is taken up by the liver, but a small proportion is taken up elsewhere (Entenman, Chaikoff & Zilversmit, 1946; Gould, 1951, 1954). There is no comparable information on the behaviour in the circulation of the unesterified fatty acid or triglyceride components of the lipoproteins.

a. Permeability of the Capillary Membrane to Lipoproteins

The passage of lipoprotein molecules across the blood capillary membrane is probably dependent on the same factors as those which determine the permeability of the membrane to other macromolecules (cf. Pappenheimer, 1953). According to present theory, the membrane behaves as a semi-permeable structure containing pores which permit the restricted diffusion of protein molecules. In this pore theory, as put forward by Pappenheimer, it was calculated that the pores had a mean diameter of 60–90 Å; but more recent work, in which dextrans of high molecular weight have been used to study capillary permeability, has indicated that, in addition to pores of this size, there may be a smaller number of so-called leaks with a calculated radius of from 116 Å to 350 Å (Grotte, 1956). The proportion of leaks to pores, and hence the permeability characteristics of the membrane, differs in various sites in the body, and there is now adequate evidence to show that large molecules leave the blood more readily in the liver than elsewhere (cf. Yoffey & Courtice, 1956).

That the lipoproteins can diffuse from the blood into the tissue fluid through such leaks or pores has been shown indirectly by the analysis of lymph. In 1953, Page, Lewis & Plahl, using the ultracentrifugal method, first identified lipoproteins in the thoracic-duct lymph of dogs in the post-absorptive state. These lipoproteins were the same as those in the plasma, but were present in lower concentrations. Lipoproteins have also been identified in the lymph from thoracic, cervical, hepatic and leg ducts of cats, dogs and rabbits by zone electrophoresis (Courtice & Morris, 1955; Morris, 1957). The electrophoretic pattern of the lipoproteins in the lymph was similar to that in the plasma, but the concentration in the lymph was found to differ at the various sites. Hepatic lymph contained lipoprotein concentrations only slightly lower than those in plasma, whereas the concentration in leg lymph was about 30% of the plasma levels.

In the blood of the various animals used in these investigations most of the lipid is present in α-lipoproteins (Morris & Courtice, 1955a). In man, on the other hand, 75% of the total lipid in the blood occurs in the β-lipoproteins (Barr,

1953). Since these are larger molecules, it is likely that the capillary membrane is less permeable to them. In rabbits a high concentration of lipoproteins with the ultracentrifugal and electrophoretic properties of the β-class can be produced in the blood by feeding a diet rich in cholesterol. The composition of the lymph in rabbits fed cholesterol has been investigated, and it has been found that β-lipoprotein is present in lymph obtained from the leg and thoracic duct (Kellner, 1954; Morris & Courtice, 1955b). Morris and Courtice found, however, that the ratio of the concentration of cholesterol and phospholipid in the blood to the concentration in lymph was greater in these animals than in normal ones, and concluded from this that the capillary membrane was less permeable to plasma lipids present as β-lipoproteins.

The output of cholesterol and phospholipid in lymph in the post-absorptive state is a measure of the amount of lipoprotein which circulates through tissue fluid and lymph. Reinhardt, Fishler & Chaikoff (1944) found that 9–20% of the plasma phospholipid could be collected from the thoracic duct lymph in 3–6 hours in dogs. Courtice & Morris (1955) calculated that in cats 58% of the plasma cholesterol and 69% of the plasma phospholipid escaped from the blood and were returned to the circulation in the lymph in 24 hours. These values are very similar to those obtained for the passage of globulin from blood to lymph (cf. Yoffey & Courtice, 1956) and this suggests that the permeability of the capillaries to lipoproteins is of the same order as to globulins. In rabbits fed cholesterol there is an increase in the amount of cholesterol returned to the blood by the lymph each day, but this represents only 25% of the cholesterol in the blood as contrasted with 50% in normal rabbits. This supports the view that the capillary membrane is less permeable to β-lipoprotein than to the smaller α-lipoprotein molecules (Morris & Courtice, 1955b).

It is possible to explain these observations on the lipoprotein content of lymph in terms of the pore theory of capillary permeability. The α-lipoprotein molecules, thought to have an equatorial diameter of 50 Å, could diffuse through pores of the size proposed by Pappenheimer. The β-lipoprotein molecules with a diameter of about 185 Å would, according to present views, escape only through the relatively infrequent capillary leaks.

b. Direct Uptake of Lipoprotein Lipid from the Blood

The rate at which the lipoproteins circulate through the tissue fluid and lymph is insufficient to account for the rate at which the cholesterol and phospholipid in the plasma come into equilibrium with the tissues. Some lipid could be exchanged between tissue cells and lipoprotein which has leaked from the blood, but the values already quoted for the circulating half-lives of cholesterol and phospholipid demand that a more rapid type of transfer must also occur. The special features of the hepatic circulation suggest that this could occur readily in the liver. It has already been pointed out that the composition of liver lymph indicates that the tissue spaces of the liver contain a higher concentration of lipoprotein than tissue spaces elsewhere. It appears that the hepatic sinusoids may not have a continuous endothelial lining, as found in capillaries elsewhere, but a fenestrated structure which permits the liver cells in places to come into immediate contact with the circulating blood (Fawcett, 1955). If the sinusoidal walls are fenestrated in this way, lipids can be exchanged between the lipoproteins of the blood and the liver cells directly (cf. Gould, 1954)—that is, under conditions not governed by considerations of capillary permeability.

The uptake of lipids by cells in the liver may actually be dependent on the uptake of labile lipid components of the lipoproteins rather than on the ingestion of the complete lipoprotein molecule, although the latter may occur to a limited extent. It has been shown that certain lipids can exchange readily between different classes of lipoprotein and between lipoprotein and red cells (Hagerman & Gould, 1951; Kunkel & Bearn, 1954); the same process may occur between liver cells and plasma lipoproteins, and to a lesser extent between tissue cells elsewhere and the lipoproteins of the tissue fluid.

There is no evidence that the "clearing reaction", discussed below, is concerned in the removal of the cholesterol or phospholipid components of the α- and β-lipoproteins from the blood. Changes in the electrophoretic mobility of the α- and β-lipoproteins may occur following the injection of heparin intravenously (Herbst & Hurley, 1954; Comfort, 1955; Dangerfield & Smith, 1955), but these changes are apparently explained by the binding of released fatty acids by these molecules (Gordon, 1955; Laurell, 1955) and there is nothing to suggest that they influence the removal of the molecules from the blood. Where the lipoproteins contain triglyceride, this may be split off in the blood by a hydrolytic mechanism which is discussed below in relation to chylomicron fat.

2. Removal of Chylomicron Lipid from the Blood

The removal of chylomicron lipid from the blood occurs in its most obvious form during the net transfer of fat from the gut to the tissues that follows a fatty meal. The chylomicrons are considerably larger than the molecules of β-lipoprotein and, with a diameter of 0.5–1 μ, each particle carries something of the order of 500 million fatty acid molecules. Nevertheless, it can be shown that fat introduced into the circulation as chylomicrons is removed extremely rapidly; indeed, much more rapidly than the lipid of the lipoproteins. It has been shown that isotopically labelled chylomicron fat, obtained by feeding dogs or rats glyceryl tri[1-^{14}C]palmitate, has a circulating half-life of the order of ten minutes when injected intravenously (Havel & Fredrickson, 1956; French & Morris, 1957). After it has left the circulation a large proportion of the fat can be recovered from the liver, although some appears to be taken up in other tissues, including the fat depots (French & Morris, 1958). It is necessary to consider two possible mechanisms for the removal of chylomicrons from the blood: either they leave the circulation as intact particles, or the particles are in some way degraded in the blood stream, and the fat changed into a more readily diffusible form.

a. Removal of Chylomicron Lipid as Particles

i. Leakage through the Capillary Membrane

The dimensions of the chylomicrons are such that it appears unlikely that they can leave the blood by diffusion through the capillary pores or leaks. Nevertheless, there is evidence that, at some sites in the body, chylomicrons may gain access to the tissue fluid and lymph. Courtice & Morris (1955) found that, although lymph obtained from the cervical and leg ducts appeared clear to the naked eye when the plasma was turbid, it was seen to contain some chylomicrons when examined by dark-ground illumination. The concentration of chylomicrons in the lymph was directly related to their concentration in the

blood. Examination of the liver lymph suggested that the leakage of chylomicrons from the circulation occurred much more readily in the liver than elsewhere. In the cat, the concentration of chylomicrons in the liver lymph was much higher than in the cervical duct; it was related to the concentration in the blood and, when this was high, the liver lymph became visibly turbid if the portal venous pressure was raised and the rate of formation of liver lymph increased (Morris & Courtice, 1956).

These experiments show that leakage of intact chylomicrons can occur; it is more difficult to assess the quantitative significance of the process. In cats, only about 0.4% of chylomicron fat introduced into the circulation appears in the liver lymph (Morris & Courtice, 1956); in rats, about 1% of intravenously injected labelled chylomicron fat appears in the lymph from the thoracic duct (French & Morris, 1957). It must be emphasized, however, that the removal of chylomicron fat from the blood, in contrast to the removal of lipoprotein lipid, occurs in conditions where there is a net transfer of lipid to the tissues; so that the concentration of chylomicrons in the lymph reflects the number which have not been taken up from tissue fluid rather than the number which reach the tissue fluid from the blood. The number of intact chylomicrons which leak into the tissue fluid may thus be greatly in excess of the number which recirculate in the lymph. The structural features of the capillary membrane which could allow this to occur in sites other than the liver are, however, unknown.

ii. Uptake by Phagocytic Activity

The possibility that chylomicrons may be removed from the blood by a process of phagocytosis has also to be considered. When various artificially prepared fat emulsions, with emulsion particles of the same size as the chylomicrons, are injected into the blood, it can be shown that they are taken up by reticulo-endothelial cells, and particularly by the Kupffer cells of the liver (Gilbert & Jomier, 1908; Jaffé & Berman, 1928). The extent to which fat particles are taken up in this way has been shown to vary with the emulsifying agent used in their preparation (Waddell, Geyer, Saslaw & Stare, 1953). This suggests that the behaviour of the fat particles is related to their surface films and that in this respect chylomicrons need to be considered as a special case. Indeed, Murray & Freeman (1951) found that, when chyle was injected intravenously, the fat appeared in liver parenchymal cells, not Kupffer cells, and, in contrast to the fat from the artificial emulsion which they used, was not taken up by reticulo-endothelial cells in the spleen. It has been shown that, when large amounts of cholesterol are fed to rats, the cholesterol enters the blood stream in chylomicrons and can subsequently be identified in Kupffer cells (Byers, Mist-St George & Friedman, 1957). This sequence of events does not, however, necessarily imply a direct uptake of intact chylomicrons by Kupffer cells. At present, the available evidence supports the view that the surface properties of chylomicrons prevent their ready phagocytosis by reticulo-endothelium. It should be noted, however, that when chylomicrons are injected into the circulation in differing concentrations their behaviour is similar in some respects to the behaviour of particles which are known to be taken up by phagocytosis (French & Morris, 1957). The removal of chylomicrons and of carbon particles, for example, follows an exponential function, but at a rate which is inversely proportional to the initial concentration. This pattern of removal is said to be characteristic of phagocytic activity (Biozzi, Benacerraf & Halpern, 1953). The possibility still exists, therefore, that chylomicrons may be taken up by phagocytosis by cells which utilize or store fat, although perhaps not by cells of the reticulo-endothelial system. The special relationship of the hepatic cells to the circulation[1] suggests that this type of removal could occur in the liver.

b. Degradation of Chylomicrons in the Blood: the Heparin-Clearing Reaction

A possible mechanism for the degradation of chylomicrons in the blood and the release of their lipid in a more readily diffusible form has been suggested by the so-called heparin-clearing reaction, first described by Hahn in 1943 (cf. Robinson & French, 1957). Following the injection of heparin into the circulation, an enzyme appears in the blood which hydrolyses the triglyceride component of the chylomicrons to glycerol and unesterified fatty acid (Shore, Nichols & Freeman, 1953; Robinson & French, 1953). The hydrolytic reaction can be studied in vitro by mixing either lipaemic plasma or fatty chyle with plasma obtained from an animal into which heparin has been injected (Anderson & Fawcett, 1950; French, Robinson & Florey, 1953; Brown, Boyle & Anfinsen, 1953). As the reaction proceeds, the originally turbid mixture becomes clear, the chylomicrons disappear, and the released fatty acid remains in solution in association with the plasma albumin (Robinson & French, 1953).

If heparin is injected when the blood already contains chylomicrons, there is an increase in the concentration of unesterified fatty acids in the blood (Lindgren, Nichols & Freeman, 1955) and an increase in the rate at which chylomicron fat is removed from the circulation (Brown, 1952; Bragdon & Havel, 1954; French & Morris, 1957). This suggests that the hydrolysis of chylomicron fat induced by heparin injection facilitates the removal of fat from the blood.

The extent to which a hydrolytic reaction analogous to the heparin-clearing reaction may operate in the blood under physiological conditions is still disputed. It has been shown that a small increase in unesterified fatty acids occurs in the plasma during alimentary lipaemia (Grossman, Palm, Becker & Moeller, 1954; Robinson, Harris, Poole & Jeffries, 1955; Spitzer & Miller, 1956), but it is still not clear how far this may be accounted for by fatty acids which enter the blood from the thoracic duct (Borgström & Tryding, 1956). The increase in the specific activity of the unesterified fatty acids in the plasma—which occurs during the time that ^{14}C-labelled chylomicron fat is being removed from the circulation (Havel & Fredrickson, 1956)—is stronger, though still not conclusive, evidence for hydrolysis of the fat in the blood. The distribution of triglyceride in the plasma during alimentary lipaemia, as shown by ultracentrifugal analysis, is compatible with the view that hydrolytic degradation of lipid particles occurs in the blood. The orderly sequence of macromolecules of decreasing triglyceride content and increasing density described by Jones, Gofman, Lindgren, Lyon, Graham, Strisower & Nichols (1951) could represent the intermediate products in the breakdown of chylomicrons by hydrolysis. At present, however, there is no information about the relationship between the rate of turnover of such molecules

[1] For a discussion of blood flow through the liver, see Andrews, *Brit. med. Bull.* 1957, 13, 82.—Ed.

and the rate at which chylomicron fat is removed from the blood.

In addition to the above evidence that hydrolysis of chylomicron fat occurs in vivo, it has been shown that the plasma of some animal species, withdrawn during the course of alimentary lipaemia, has the ability to hydrolyse chylomicron fat in vitro (Jeffries, 1954). This phenomenon is of considerable interest, since it suggests that a clearing reaction can be initiated in the blood without the injection of heparin. This reaction appears to be identical with the one induced by small amounts of heparin, but at present there is no evidence that it could account for the hydrolysis of more than a proportion of the fat entering the blood (Robinson, Jeffries & French, 1954).

So far, endogenous heparin has not been directly implicated in a physiological clearing reaction in the blood. The evidence which links the heparin-clearing reaction with removal of chylomicron fat is based on the effect in vivo of certain substances which are known to inhibit the heparin-clearing reaction in vitro. Thus, a number of those inhibitors, when injected intravenously, will delay the removal of chylomicrons from the blood (Brown, 1952; Bragdon & Havel, 1954; Robinson *et al.* 1954; French & Morris, 1957). Particular interest attaches to the observation that protamine—a known antagonist of heparin—has this effect. It must be realized, however, that these substances may have effects in vivo which are not related to their inhibition of lipolysis. Many of the substances used are surface-active and, by altering the surface properties of the chylomicrons, may affect the behaviour of chylomicrons in other ways. It has been found, for example, that, after the injection of the surface-active agent Triton WR-1339, some uptake of chylomicrons occurs in reticulo-endothelial cells in the spleen (French & Morris, 1958). Under these circumstances the chylomicrons behave like the particles of artificially prepared emulsions which are removed from the blood more slowly than normal chylomicrons.

In summary, there is at present no evidence that chylomicrons are normally removed exclusively either as particles or by hydrolysis. It is more probable that both mechanisms occur and are complementary to each other. Removal of particles can apparently still occur when hydrolysis in the blood is inhibited (French & Morris, 1957) and it occurs in the isolated perfused liver under conditions in which prior hydrolysis has, as far as possible, been excluded (Morris & French, 1958). On the other hand, it appears that an enzyme with the properties of clearing factor must have a function in lipid transport. Until more is known, however, about the site of its production and its activation in the absence of injected heparin, its contribution to the removal process must remain uncertain (cf. Korn, 1955; Korn & Quigley, 1957; Robinson & Harris, 1958).

3. Removal of Unesterified Fatty Acids from the Blood

Unesterified fatty acids presumably occur in the blood as soluble complexes with the plasma albumin (Lindgren *et al.* 1955). In addition to the increase in concentration of unesterified fatty acid which may occur from intravascular hydrolysis of chylomicron fat, unesterified fatty acid may enter the blood from the lymph (Borgström & Tryding, 1956) or be mobilized in this form from the fat depots (Dole, 1956; Gordon & Cherkes, 1956). It is also possible that a large part of the lipid which passes from the liver to other tissues via the blood does so in this form (cf. Harper, Neal & Hlavacek, 1953).

Unesterified fatty acids are removed very readily from the circulation. Havel & Fredrickson (1956) found that when labelled fatty acids were injected intravenously into dogs they had a circulating half-life of about two minutes; Laurell (1957) obtained similar results in man. This rate of passage of fatty acid molecules across the capillary membrane is many times greater than the rate of passage of the albumin molecules with which they are associated in the blood (cf. Wasserman & Mayerson, 1951). It seems possible, therefore, that the association serves only to keep the fatty acids in solution, and that the fatty acid–albumin complex dissociates at the surface of the endothelial cells in contact with the blood. The fatty acids may then be able to cross the capillary membrane through the endothelial cells rather than by diffusion through pores. Such a mechanism has already been proposed to account for the high permeability of capillaries to small lipid-soluble molecules (Renkin, 1953). Liver cells can probably take up unesterified fatty acids directly from the blood at a high rate, since it has been shown that in the isolated and perfused rat liver unesterified fatty acids are taken up and oxidized more readily than chylomicron triglyceride (Morris & French, 1958). There is also evidence that fat cells isolated from the fat depots take up unesterified fatty acids directly (Shapiro, 1957). However, the uptake of fatty acids by these cells in the living animal is probably dependent on the prior transport of fat across the capillary membrane, since fat cells are not thought to be in direct contact with the blood.

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HORMONAL CONTROL OF THE CIRCULATING LIPIDS

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1 Physical characteristics of the circulating lipids
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The hormonal control of lipid metabolism has been less fully investigated than the hormonal control of carbohydrate or protein metabolism. Much of the knowledge concerning the hormonal influences of the circulating lipids has been obtained from studies designed to throw light on the aetiology of coronary artery disease. It is widely believed that there is a close association, but not necessarily a causal relationship, between abnormalities in the character and concentration of the circulating lipids with the development of coronary atheroma. These abnormalities, which have been recently reviewed (Oliver & Boyd, 1957a), include elevation of the circulating cholesterol, elevation of cholesterol:phospholipid ratio (C:P ratio), and elevation of the β-lipoprotein cholesterol and certain low-density lipoproteins. The recognition of the association of these abnormalities with coronary disease has resulted in numerous studies of the circulating lipids. It is the aim of this paper to summarize some of the hormonal influences and to consider the possible pathways of hormonal control of the circulating lipids, but first it is necessary to consider some of their physical characteristics.

1. Physical Characteristics of the Circulating Lipids

Although normal plasma during fasting is translucent, it contains several hundred milligrams of lipid material in each hundred millilitres. The plasma lipids are principally phospholipids, esterified cholesterol and unesterified or free cholesterol, and there are also triglycerides and fat-soluble vitamins. These hydrophobic molecules are rendered water-soluble by combination with α- and β-globulins, thus forming conjugated proteins which are referred to as the α- and β-lipoproteins. With the exception of chylomicra, which are largely triglycerides, almost all the plasma lipids can be accounted for as α- and β-lipoproteins. These loose complexes of unknown structure have widely different physical characteristics which allow their separation according to differences in electrical charge, hydrated densities or molecular weight. The plasma lipids are often considered either collectively or individually as homogeneous entities: for example, plasma cholesterol is quoted in terms of its total concentration, but, in fact, the plasma cholesterol is partitioned between the two major lipoproteins, which may themselves consist of several entities. As the plasma α- and β-lipoproteins consist of complexes of cholesterol, phospholipid and protein, the measurement of the plasma total cholesterol or the C:P ratio is dependent on the concentration and ratio of the plasma lipoproteins, and these three separate measurements should be regarded as dependent variables.

2. Effects of Hormones on the Circulating Lipids in Man

a. Physiological Hormonal Influences

Studies of the circulating lipids during the menstrual cycle in healthy young women have shown that the plasma cholesterol, the plasma C:P ratio and the β-lipoprotein cholesterol are lower at ovulation than at any other time during the cycle (Oliver & Boyd, 1953a, 1955b). No similar regular cyclical depression of these lipids was observed in healthy young men. Although at the present time most of the assay methods for sex hormones are only qualitative or semi-quantitative, oestrogens have been assayed with greater precision than others, and Brown (1955) has confirmed that the secretion of oestrogens is maximal just before ovulation. It has been suggested that the depression of the blood lipids at ovulation could result from the increased endogenous secretion of oestrogens at that time.

In pregnancy, there is elevation of the plasma cholesterol, the C:P ratio and the β-lipoprotein cholesterol during the last trimester (Oliver & Boyd, 1955a). These changes may be related to alterations in the endogenous hormonal balance, but there are certain paradoxes in so far as hypercholesterolaemia occurs at a time when there is increased oestrogen, increased adrenocortical and increased thyroid activity, and each of these independently produces the opposite effect.

Elevation of the plasma cholesterol has been observed during the first post-menopausal decade in healthy women (Oliver & Boyd, 1953b; Adlersberg, Schaefer, Steinberg & Wang, 1956; Oliver & Boyd, 1958), and it has been suggested that this rise may be explained on an endocrine basis and related to the decrease in oestrogenic activity following the menopause.

b. Pathological Hormonal Influences

In certain endocrine diseases there is elevation of the circulating cholesterol and of other lipids. It has long been known that there is hypercholesterolaemia in myxoedema, but there is no direct correlation between the height of the plasma cholesterol with retardation of the basal metabolic rate (Dodds & Robertson, 1933; Lerman & Pitt-Rivers, 1955). In diabetes, when there is hyperglycaemia there is usually hypercholesterolaemia, but these values are again not strongly correlated. It has not been clearly established that the hypercholesterolaemia present in association with uncontrolled diabetes is directly related to insulin lack, since renal damage and vascular disease could contribute to its development. Hypercholesterolaemia has been observed in some patients with Cushing's disease (Heinbecker & Pfeiffenberger, 1950), but this does not seem to be a consistent finding and might be explained by the accompanying diabetes and renal damage. Hypercholesterolaemia not uncommonly occurs after bilateral ovariectomy (Robinson, Higano & Cohen, 1957; Oliver &

Boyd, 1958) and also has been reported after orchidectomy performed in healthy men for homosexuality (Teilum, 1937) or mental deficiency (Hamilton, Bunch, Mastler & Imagarva, 1956).

Reduction of the circulating cholesterol occurs with overactivity of the thyroid and has also been reported in 40% of a series of 53 patients with Addison's disease (Marañon & Benitez, 1933). In eunuchs there is hypocholesterolaemia and an increase in high-density lipoproteins (Furman & Howard, 1957).

c. Influence of Administered Hormones

With the possible exceptions of somatotrophin and prolactin, most of the hormones of the anterior pituitary have only one target organ and, in the absence of this target organ, no significant metabolic effect has been demonstrated. Thus the influences of anterior pituitary hormones on the blood lipids are regarded as comparable with the effects of the hormones secreted by the respective target organs. In our experience the administration of beef or porcine somatotrophin preparations to man produced no significant effect on the circulating cholesterol (Oliver & Boyd, 1956b), but this conclusion may need to be revised when human growth hormone is available.

Adrenocorticotrophin (ACTH) and cortisone acetate lower the plasma cholesterol, the plasma C:P ratio and the β-lipoprotein cholesterol in hypercholesterolaemic subjects (Conn, Vogel, Louis & Fajans, 1950; Oliver & Boyd, 1955b, 1956b). Adlersberg, Schaefer & Drachman (1950) have shown that both ACTH and cortisone caused elevation of initially low serum cholesterol levels in patients severely ill with collagen diseases, and Mann & White (1953) have interpreted this rise as due to the removal of stress during the acute phase of the severe illness.

It has been known for many years that thyroid extract lowers the plasma cholesterol and more recently thyroxine has been shown to reduce the β-lipoprotein concentration and the concentration of certain low-density lipoproteins as determined in the ultracentrifuge (Malmros & Swahn, 1953; Strisower, Gofman, Galioni, Almada & Simon, 1954). The oral administration of the acetic acid analogue of thyroxine and of 3: 5: 3′-triiodo-*l*-thyronine and its acetic acid analogue lowers the plasma cholesterol, the C:P ratio and β-lipoprotein cholesterol in both hypothyroid and euthyroid subjects (Lerman & Pitt-Rivers, 1955; Trotter, 1955; Goolden, 1956; Trotter, 1956; Boyd & Oliver, 1957; Oliver & Boyd, 1957b). These analogues, together with 3 : 5-diiodo-*l*-thyronine, all lower the plasma cholesterol without apparently elevating the basal metabolic rate. This dissociation of action is transient and dependent upon the administration of a specific dose.

The administration of oestrogens to women after the menopause has resulted in a decrease in the circulating cholesterol, with elevation of the phospholipids (Eilert, 1949, 1953). This action of oestrogens, which also depressed the β-lipoprotein cholesterol, has been confirmed in hypercholesterolaemic men (Barr, 1953; Oliver & Boyd, 1954, 1956a). In contrast, androgens cause elevation of these values (Barr, 1953; Oliver & Boyd, 1956a). The intramuscular administration of progesterone did not result in any significant change in the circulating blood lipids (Oliver & Boyd, 1956a). It is well known that the administration of insulin in diabetes is associated with reduction of hyperglycaemia and also a reduction of any associated hypercholesterolaemia, but there is no definite evidence to show that insulin administered to metabolically normal men (with mental disease) has any significant effect on the blood lipids. The intravenous infusion of glucagon to healthy men resulted in immediate elevation of the blood sugar, and after a few days there was slight elevation of the circulating cholesterol, which was of doubtful significance (Oliver & Boyd, 1956b).

The parenteral administration of adrenaline did not cause any significant change in the plasma cholesterol (Dury & Treadwell, 1955).

These various hormonal influences on the blood lipids in man are summarized in Chart I.

CHART I. SUMMARY OF SOME OF THE HORMONAL INFLUENCES ON CIRCULATING CHOLESTEROL

Hormones and conditions that raise plasma cholesterol	Hormones and conditions that lower plasma cholesterol
Menopause	Thyrotoxicosis
Pregnancy	ACTH
Diabetes	Cortisone
Myxoedema	Thyroxine
Bilateral ovariectomy	Oestrogens
Androgens	

3. The Nature of Hormonal Control of the Circulating Lipids

The hormonal control of the blood lipids presents a different problem from the hormonal control of lipogenesis. The hormonal control of lipogenesis is concerned with adjustment of the rates of intracellular synthesis of fatty acids and cholesterol. By contrast, the hormonal control of the circulating lipids is concerned with maintenance of the plasma concentration of these lipids. Since there is no evidence for the oxidative catabolism of lipids in the extracellular fluid, the concentration of the circulating lipoproteins would appear to be dependent upon three principal factors. First, changes in the rates of transference between the extracellular and intracellular lipoproteins, such as can result from alteration in the rate of lipid synthesis or degradation, will alter the concentration of the circulating lipoproteins. Second, any factor which influences the distribution of lipoproteins between the plasma and the interstitial fluid, that is at the level of the capillary endothelium, will alter the plasma lipoprotein concentrations. Third, physiological changes in the plasma volume resulting from alterations in the degree of hydration of this compartment will obviously influence the circulating lipoprotein concentrations. Hormones could affect the concentration of plasma lipoproteins by influencing any or all of these factors.

The circulating cholesterol originates largely from hepatic biosynthesis and to a lesser extent from dietary and extrahepatic sources; the rate of intracellular hepatic synthesis from non-sterol precursors, such as active acetate, varies considerably under physiological conditions. The newly synthesized sterol is passed into the plasma where it mixes with a large reserve of circulating cholesterol and ultimately equilibrates with cholesterol outside the plasma. If this cholesterol "pool" is to remain of constant size, then the rates at which cholesterol enters and leaves the pool must be identical. Thus, in man, cholesterol synthesized in liver exchanges with a cholesterol "pool" of several grams and, because of the large

size of this reserve, a small change in the rate of hepatic synthesis of cholesterol over a short period might not be reflected by any significant change in the concentration of circulating cholesterol; for example, starvation results in inhibition of hepatic cholesterologenesis with little immediate effect on the plasma cholesterol. Nevertheless, it is possible that hormones which produce any marked or lasting effect on intracellular lipid anabolism or catabolism may ultimately affect the concentration of circulating lipoproteins.

The plasma lipoproteins are probably in a dynamic equilibrium with the lipoproteins of the interstitial fluid and of the cells, and it is unfortunate that plasma is the only body-fluid readily accessible for sampling. Whereas the composition of plasma is fairly constant throughout the circulation, the composition of interstitial fluid is very variable. This fact, coupled with the technical difficulty in obtaining lymph samples, has prevented the simultaneous analysis of plasma and lymph except in a few isolated instances. Since we can measure the lipoproteins only in plasma, the mechanism of any change in the concentration of these lipoproteins will remain obscure until their distribution between cells and the interstitial fluid on the one hand, and the interstitial fluid and plasma on the other, is clearly established. The problem is further complicated by the labile nature of the lipoprotein molecule in which the lipid and protein moieties may "turn over" at different rates. This dynamic exchange of lipid at a membrane could be due to lipoprotein exchange through, or lipid exchange between, different protein molecules.

At present, no conclusions should therefore be drawn concerning the mechanism of action of any hormones which produce changes in the concentration of the plasma lipoproteins, but it is both necessary and desirable to explore the effect of hormones on the plasma lipoprotein concentrations. The oral and parenteral administration of hormones to man, followed by determination of the plasma lipoprotein concentration, is the limit to which most human studies can be taken.

Unfortunately, the common domestic and laboratory species of animals respond to certain hormones in a different fashion from man. One reason for this species variation may be the different concentrations of α- and β-lipoproteins in man and in animals; the β-lipoprotein predominates in primates, whereas the α-lipoprotein predominates in most other species (cf. Morris & Courtice, 1955). Since hepatic cholesterol biosynthesis is usually measured by isotopic methods, most studies on factors influencing the rate of biosynthesis have been confined to laboratory animals. Although it has been possible to study simultaneously the effect of hormones on the plasma cholesterol levels and on synthesis in the liver in certain species, no clear-cut relationship has emerged between the plasma cholesterol level and the rate of biosynthesis of cholesterol.

It has already been seen that in man thyroid, oestrogenic and adrenocortical hormones lower hypercholesterolaemia, while androgens cause elevation of the circulating cholesterol (Chart I). Some of the potential sites of action of hormones which alter the concentration of the circulating lipids have been discussed, and now the possible actions of these specific endocrine secretions will be considered briefly in relation to certain experimental observations.

It is well known that in man reduction of thyroid function elevates the plasma cholesterol, whereas hypocholesterolaemia exists when thyroid function is increased. While this suggests an inverse relationship between oxygen consumption and plasma cholesterol levels, this relationship probably exists only with extremes of thyroid imbalance and does not apparently hold for euthyroid subjects. Similarly, in the rat thyroidectomy causes a marked fall in metabolic rate and only a slight increase in plasma cholesterol, whereas hyperthyroidism (induced by administration of thyroxine and analogues) is associated with marked elevation of the metabolic rate and little or no effect on the circulating cholesterol level. Despite this weak correlation between the concentration of the circulating lipids with oxygen consumption, there is a positive relationship between hepatic cholesterol biosynthesis and oxygen consumption (Boyd, unpublished observations). There is evidence (Barker, 1956) that some thyroid analogues selectively increase the oxygen consumption of certain organs, particularly that of the liver. Since the only organ which contributes significantly to the plasma-cholesterol "pool" is the liver, this action of thyroid analogues could result in a differential effect on cholesterol metabolism. In view of the speed with which certain thyroxine analogues can reduce the plasma cholesterol in myxoedematous patients and myxoedematous animals, it would seem probable that another action of this hormone may be to influence the cholesterol distribution between the body-fluid compartments.

The cholesterol-lowering action of oestrogens is slower than the depression caused by thyroxine analogues. It is associated with a mild haemodilution, which is insufficient to account for the decrease in concentration of the circulating cholesterol. The mechanism of this depression in man is still obscure, but in the rat certain oestrogenic substances also cause marked depression of the plasma cholesterol (Levin, 1945), with associated inhibition of hepatic cholesterol biosynthesis (Boyd & McGuire, 1956). In contrast, oestrogens cause elevation of the plasma cholesterol in rabbits and fowls (Lorenz, Chaikoff & Entenman, 1938), and this difference in action is obscure. At present, it has not been established whether the depressant effect of the oestrogen is a direct action, or whether it is mediated indirectly through the anterior pituitary.

The decrease in the concentration of circulating cholesterol caused by ACTH and cortisone cannot be explained by the associated increase in plasma volume, but whether an increase in capillary permeability contributes to this effect is not known. In the rat, adrenalectomy does not influence the rate of hepatic cholesterol biosynthesis and results only in a slight fall in plasma cholesterol.

It is well known that androgens have a pronounced anabolic action resulting in protein and nitrogen retention. As the plasma cholesterol is present in the cholesterol "pool" as a conjugated protein, the protein retention action of androgens may help to explain the elevation of plasma cholesterol.

4. Summary

Hormones influence the circulating lipids physiologically, in endocrine diseases and following administration.

The concentration of circulating lipids is dependent upon changes in hepatic cholesterol biosynthesis, degradation and excretion, interchanges between the plasma and the interstitial fluid, and changes in the plasma volume. Hormones may affect the concentration of the circulating lipids by influencing any of these parameters.

At present, there is insufficient evidence to reach definite conclusions concerning the mechanism of the effect of any hormone on the circulating lipids.

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THE EFFECT OF DIETARY FATS ON THE BLOOD LIPIDS AND THEIR RELATION TO ISCHAEMIC HEART DISEASE

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2 Effect of different fats on the serum cholesterol levels
  a Mechanism of action of oils of high iodine value
  b Metabolic functions of the essential fatty acids
3 Applications to theories on the pathogenesis of ischaemic heart disease
  a Relationship of coronary atherosclerosis to ischaemic heart disease
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Ischaemic heart disease is the commonest cause of death at present in most parts of the British Commonwealth, North America and in other western industrialized areas, yet before 1920 the disease was a rarity. For example, in Britain during 1956 over 45,000 males died from this cause, yet in 1921 the figure was only 743. It is true that, with improved public health measures, an increased number are surviving to the age at which the disease is common, but over the last ten to fifteen years the death-rate as standardized by age has been doubled and at some ages even trebled (Martin, 1956). Improvements in diagnosis and longer survival, while they probably contribute, can hardly account for the whole of these recent trends.

Not long ago ischaemic heart disease was held to be a phenomenon of ageing, and survival was thought to depend largely upon inherited anatomical and mechanical factors within the coronary arterial tree. There was much in favour of this view. There was a familial incidence, it was a disease common in people derived principally from Anglo-Saxon stock, and it was a frequent complication of other inherited disorders such as essential hypercholesterolaemia and diabetes mellitus. A great deal of attention was paid to certain constitutional factors and it was claimed that mesomorphic individuals were more susceptible than ectomorphs in a given community (Gertler & White, 1954).

Members of a family or a given population group are usually subjected to similar environmental influences. Body-build may be influenced partly or largely by the mode of life in childhood, and social and occupational class by that of one's forebears, so that in practice it is not easy to differentiate those factors contributed by heredity from those occurring in the environment. Often both kinds of factor are involved, and the extent to which the one is overborne by the other becomes a vital issue with respect to ischaemic heart disease.

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1. Development of the Dietary Fat Hypothesis

Although susceptibility may be, in part, genetically determined, extension of epidemiological research into the field of non-infectious disorders has focused attention on the contribution of factors in the environment. Of these, the modern sophisticated diet of western nations is at present the most suspect, and the evidence for this needs the closest examination.

For nearly half a century a relationship has been known between the diet, the serum cholesterol level and atherosclerosis in the experimental animal (Anitschkow, 1933); whether such correlation exists in man is a matter of much controversy. Although in certain animal species additional agents (for example, thiouracil) have to be administered, no lesion resembling atherosclerosis can be produced other than by dietary manipulation. Application to man soon proved a failure. Cholesterol feeding, even in massive doses, failed to affect serum cholesterol levels (Messinger, Porosowska & Steele, 1950), and many reasons were put forward to explain why the experimental animal was not comparable with man.

Probably the greatest single factor that again drew attention to the diet in man was the sharp fall in death-rate from circulatory diseases during the 1939–45 war in many European countries (Malmros, 1950; Strøm & Jensen, 1951). This was accompanied by a fall in consumption of milk, butter, cheese and eggs. There was a fall in incidence of severe atherosclerosis in Finland (Vartiainen & Kanerva, 1947), and changes in serum cholesterol levels were reported from severely rationed areas (Brull *et al.* 1945; Gsell, 1948). In Germany, a rise in serum cholesterol level in groups studied in 1947 and again in 1949 was attributed to an increased intake of fat (Schettler, 1950). Many other environmental changes occurred during this period, however.

In England, the occupational mortality statistics for the decade ending 1951 (Registrar-General, 1954) also pointed to an environmental factor. The disease was nearly twice as common in the professional and executive classes as in the labouring classes. Prosperity appeared to be the background to these social class differences, as the same trend occurred in retired men and wives over 65 years, while similar differences between rich and poor men in Madrid had been noted by Keys (1956a).

In 1952, Keys initiated a series of studies on races with wide differences in prevalence of ischaemic heart disease. By 1956 Keys (1956a) was able to show that in areas where the percentage of calories consumed as fat was about 40%, severe atherosclerosis was common and the serum cholesterol rose sharply with age. In groups eating little fat, such as the South African Bantu (Walker & Arvidsson, 1954; Bronte-Stewart, Keys & Brock, 1955), the Chimbu of New Guinea (De Wolfe & Whyte, 1958), and the Japanese (Keys, Kimura, Kusukawa, Bronte-Stewart, Larsen & Keys, 1958), severe atherosclerosis was rare and the rise in serum cholesterol with age was insignificant. At birth, Bantu and European serum cholesterol levels are the same (Bersohn & Wayburne, 1955). Differences appear early in life, and by the age of 20 significant differences are seen not only between races but between income classes

within each race (Bronte-Stewart, 1957), so that it is unlikely that these differing age trends are explicable on genetic grounds.

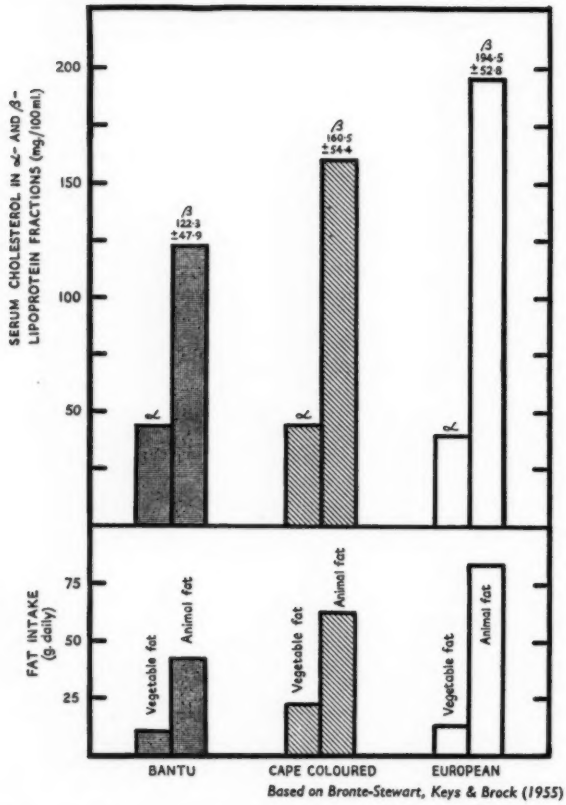
The incidence of severe atherosclerosis, too, is different in the same race living under different environmental conditions. It is far higher in Japanese who have emigrated and come under the influence of the American mode of life in Hawaii and California than in those who have remained in Japan (Larsen, 1957). Differences in mean serum cholesterol levels and dietary fat intakes run in parallel (Keys *et al.* 1958). Toor, Katchalsky, Agmon & Allalouf (1957) and Brunner & Löbl (1957) record similar differences between early and recent immigrant Yemenite Jews in Israel.

The mortality experience of the multi-racial community resident in Cape Town makes it unlikely that climatic or geographical environmental variables play any part. At the one extreme lie the Bantu in whom ischaemic heart disease is a rarity, at the other the highly susceptible European, with the Cape Coloured, mainly of mixed Malay and European stock, intermediately situated. In this order run differences in the total serum cholesterol, its distribution between the lipoprotein fractions and dietary fat intakes (see fig. 1).

In these epidemiological studies, in races widely divergent in traditional custom and mode of life, and in the same race subjected to different environmental conditions, susceptibility to severe atherosclerosis and ischaemic heart disease appears to be determined largely by environmental factors. Of the many studied, only one—i.e., dietary fat intake—has remained consistent throughout. In fact an association between diet, plasma cholesterol and atherosclerosis has emerged in man but, unlike that seen in the experimental animal, emphasis has been laid on the total fat intake instead of the dietary cholesterol intake.

In the Cape Town survey, however, the rise in fat intake from Bantu, through Coloured to European, was due to a rise in consumption of animal fat. No matter how the sample was subdivided, whether it were on economic privilege (see fig. 2), physical activity or extent of alcohol consumption, the

FIG. 1. SERUM CHOLESTEROL CONCENTRATION, AND INTAKE OF FAT, IN MULTI-RACIAL COMMUNITY RESIDENT IN CAPE TOWN



Differences in mean total cholesterol concentration of the serum in middle-aged Bantu (166 mg./100 ml.), Cape Coloured (204 mg./100 ml.) and European (234 mg./100 ml.), all resident in the same area, are associated with differences in cholesterol distribution between the lipoprotein fractions. Differences in fat consumption (Bantu, 17% of total calories; Cape Coloured, 25%; and European, 37%), which run in parallel, are due essentially to differences in animal fat consumption.

Total cholesterol in β-lipoprotein fractions for Bantu, Cape Coloured and Europeans, respectively: 72%, 77% and 83%. Results based on determinations in 364 men (132 Bantu, 118 Cape Coloured and 114 Europeans).

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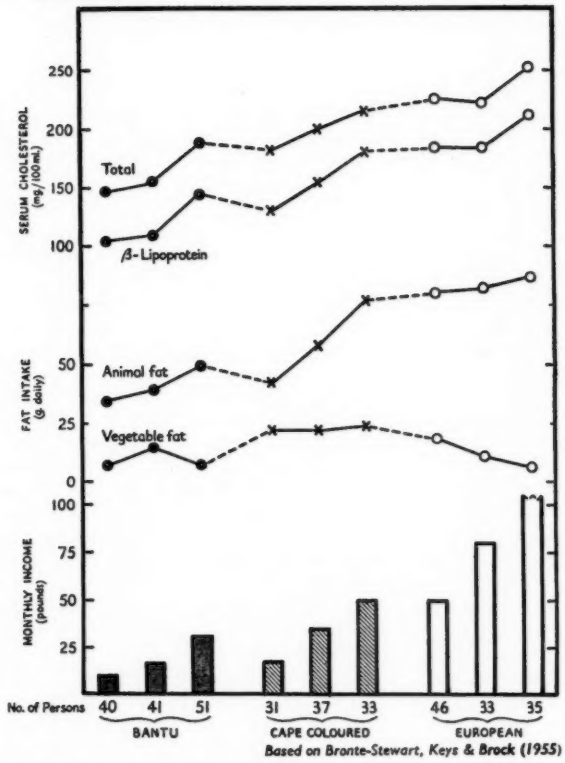
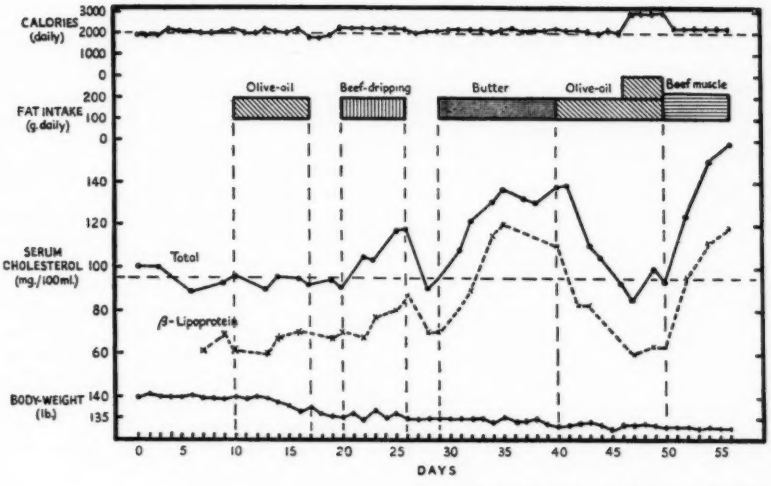
FIG. 2. RELATION OF SERUM CHOLESTEROL LEVEL TO INTAKE OF FAT IN MULTI-RACIAL COMMUNITY



The serum cholesterol levels are similar in different racial groups at the same economic level. The vegetable fat intake does not correlate with the serum cholesterol trends.

FIG. 3. EFFECT OF DIFFERENT FATS ON THE SERUM CHOLESTEROL LEVELS IN A MALE BANTU AGED 37



Based on Bronte-Stewart, Antonis, Eales & Brock (1956)

Olive-oil fails to elevate or maintain a raised serum cholesterol level. Beef-dripping, butter and beef muscle lead to immediate rises within 48 hours.

serum cholesterol trends and animal fat consumption levels remained in parallel and the intake of vegetable fat showed no correlation whatever (Bronte-Stewart, Keys & Brock, 1955).

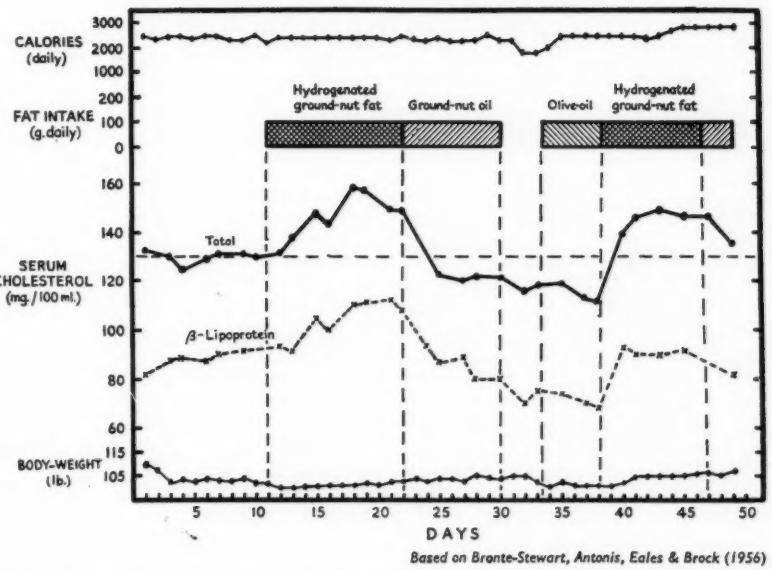
Parallelism of trends need not indicate causal association, and certainly no great reliance can be placed on dietary surveys. In epidemiological surveys too, many variables operate simultaneously and often cannot be separated. It became necessary therefore to determine whether the level of serum cholesterol was indeed influenced by the nature of the dietary fat intake.

2. Effect of Different Fats on the Serum Cholesterol Levels

That all fats did not behave in a similar fashion had been suggested by studies on vegetarians (Hardinge & Stare, 1954) and by feeding experiments (Groen, Tjiong, Kamminga & Willebrands, 1952; Kinsell, 1954). This was vehemently denied from other quarters (Keys & Anderson, 1954; Mayer, Connell, De Wolfe & Beveridge, 1954). Strictly controlled feeding experiments became necessary. Almost simultaneously, from centres as widely scattered as New York (Ahrens, Tsaltas, Hirsch & Insull, 1955; Ahrens, Hirsch, Insull, Tsaltas, Blomstrand & Peterson, 1957), California (Kinsell & Michaels, 1955), Ontario (Beveridge, Connell & Mayer, 1956), Sweden (Malmros & Wigand, 1955, 1957) and Cape Town (Bronte-Stewart, Moodie, Antonis, Eales & Brock, 1955; Bronte-Stewart, Antonis, Eales & Brock, 1956), came reports each confirming the other, although different techniques were used. Animal fats such as butter, beef-dripping or tallow, beef-muscle fat, lard and eggs led to a prompt rise in serum cholesterol levels, whereas vegetable oils, such as those from corn, sunflower, safflower, peanut and olive, lowered them. Changes could be seen within 48 hours (fig. 3) and usually reached their maximum in two to three weeks.

When these oils were fed in the hydrogenated form, their ability to decrease serum cholesterol was lost (fig. 4). The difference therefore was not merely one of animal versus vegetable. Furthermore, tropical oils, such as coconut and palm, behaved like the animal fats; and marine animal oils, if not hydrogenated, had properties of lowering cholesterol. Of interest, more with respect to the everyday diet, was the ability of vegetable oils to neutralize the effects of animal fats when both were fed together (fig. 5), and longer-term studies later revealed that, as long as these oils were consumed, their effects were maintained. Although individuals varied in the degree of response, these responses were similar in apparently normal persons, in patients with ischaemic heart disease, in individuals with essential hypercholesterolaemia, in people with initial low levels of serum cholesterol, and in subjects with differing ages, sexes and racial backgrounds.

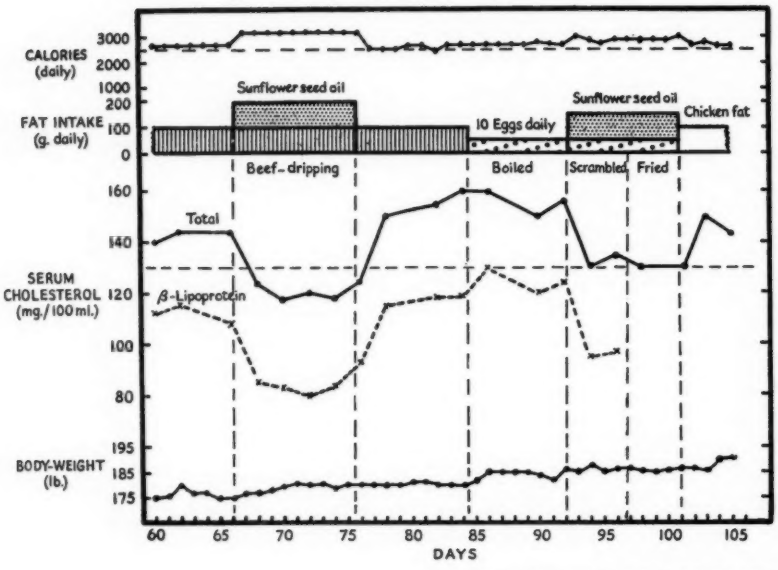
FIG. 4. EFFECT OF HYDROGENATED FATS ON THE SERUM CHOLESTEROL LEVELS IN A MALE BANTU AGED 65



Based on Bronte-Stewart, Antonis, Eales & Brock (1956)

The effect of hydrogenated ground-nut oil on the serum cholesterol level is quite different from that of the natural oil.

FIG. 5. EFFECT OF VEGETABLE OILS AND ANIMAL FATS, WHEN FED TOGETHER, IN A MALE BANTU AGED 37



Based on Bronte-Stewart, Antonis, Eales & Brock (1956)

Qualitative changes in the fat intake are more important than quantitative changes with respect to the effects on the serum cholesterol levels.

Changes in calorie, protein, sterol or vitamin intake did not appear to influence the expected response. Carbohydrate feeding to the extent of doubling the caloric intake to over 4,000 calories led to marked gains in weight, with insignificant changes in serum cholesterol in Cape Town. No significant decreases were seen after feeding tocopherol and there is no sitosterol in marine oils or cholesterol in processed vegetable or tropical oils. The difference in effects of animal and processed vegetable fats on the one hand, and natural vegetable and marine oils on the other, must lie in the constituent fatty acids or a factor at present inseparable from them. Liquid oils, with an iodine value above approximately 90, that is, containing a high proportion of the poly-unsaturated fatty acids, had cholesterol-lowering properties. The converse also held true (Table I). The feeding of the linoleate fraction isolated from sunflower-seed oil in Cape Town supplied proof of this, while the saturated fraction consisting mainly of ethyl palmitate increased the serum cholesterol levels. Stearate had insignificant effects. Horlick & Craig (1957) of Saskatchewan had the same experience, but Beveridge, Connell & Mayer (1957)—who fed fractions obtained by molecular distillation of oils—felt that some factor other than linoleate was also exerting an effect. Keys, Anderson & Grande (1957), in supporting this contention, claim that corn-oil, with its lower iodine value, was slightly more effective than sunflower-seed oil in lowering the serum cholesterol. It is very possible that this effect could be due to the greater concentration of tocopherols and other antioxidants in corn-oil, which would preserve the poly-unsaturated fatty acids so that these would reach the intestine in an unoxidized state. Doubtless these issues will be resolved when pure fatty acids become available in amounts sufficient to carry out feeding experiments on a larger number of people than has so far been possible.

a. Mechanism of Action of Oils of High Iodine Value

A simple withdrawal of saturated fatty acids from the diet could hardly explain the fall in serum cholesterol levels. Normal fat absorption accompanies the changes (Gordon, Lewis, Eales & Brock, 1957), and the oils can depress the serum cholesterol to a level lower than that attained on a fat-free diet. In the low-fat rice diet used by Hatch, Abell & Kendall (1955) the fall in serum cholesterol was associated with visible lipaemia, a rise in plasma triglycerides and an abnormal ultracentrifugal pattern in one-fifth of their patients. Ahrens and his group (1957) experienced a similar rise in triglycerides when the proportion of calories from fat was reduced to 10%. This has not been seen when the oils of high iodine value have been fed. Finally, the sharp increase in the faecal sterols and bile acids—the breakdown products of cholesterol—on feeding with these oils after a fat-free diet (Gordon *et al.* 1957) suggests an increased rate of elimination of cholesterol from the body. The reverse effect, namely the elevation of serum cholesterol levels after feeding with the more saturated fats, could be due as much to a slower elimination as to an increased synthesis of cholesterol for the transport of the more saturated fatty acids. With the fall in serum cholesterol, an increased proportion of poly-unsaturated fatty acids appears in cholesteryl esters (Antonis, 1958; Lewis, 1958). The free cholesterol alters little on feeding with the oils of high iodine value. Such changes in cholesteryl esters are of great interest in atheroma, when one notes that, in animals, tissue lipid deposition is enhanced on feeding with fats with low iodine values (Hartroft, 1955), and in essential fatty acid deficiency a marked increase in cholesterol ester deposition occurs (Alfin-Slater, Aftergood, Wells & Deuel, 1954; Basnayake & Sinclair, 1956). In tissue cultures of endothelium of human aorta, cholesterol-induced lipid deposition could be inhibited by linoleic acid and enhanced by stearic acid (Rutstein, Ingenito, Craig & Martinelli, 1958). In Cape Town, Lewis (1958) has found that the proportion of saturated plus oleic acids in cholesteryl esters of atheromatous human coronary arteries was much higher than that in the circulating plasma. In the plasma, this proportion was highest in Europeans who have had myocardial infarction and lowest in the Bantu. The values from plasmas of unaffected Europeans fell in between these two extremes (Antonis, 1958; Lewis, 1958).

b. Metabolic Functions of the Essential Fatty Acids

The "essential" role of the poly-unsaturated fatty acids in man is very controversial. The isotope-labelling studies of Lipsky, Haavik, Hopper & McDivitt (1957) suggest that man is incapable of synthesizing these acids; but a syndrome in man, acceptable as being due to essential fatty acid deficiency,

TABLE I. FATS TESTED IN MAN FOR THEIR EFFECT ON SERUM CHOLESTEROL LEVELS

The effect on serum cholesterol levels of fats thus far tested in man appears to depend upon their respective iodine numbers. With the exception of butter and coconut oil, which have short-chain fatty acids in addition, all the fats listed have long-chain fatty acids. Their average fatty acid compositions (compiled from Bailey (1945) and Hilditch (1956)) are shown, but these vary widely.

Fats raising serum cholesterol

Source or nature of fat	Iodine no. (range)	Fatty acid composition (%)			Reference
		Saturated	Monoethenoid	Polyethenoid	
Coconut	7–10	91	7	2	a, i, j
Cow's milk	26–45	58	38	4	a, b, c, g, h
Beef muscle	30–33	—	—	—	c
Beef tallow	38–50	48	50	2	a, c, b
Cocoa butter	35–40	60	37	3	a
Palm	49–57	49	41	10	a
Lard	50–90	33	54	13	a, b
Egg (hen)	68–90	31	54	14	a, c
Chicken	60–100	23	53	23	a, c, b
Hydrogenated coconut	1–3	97	3	0	a, f
Hydrogenated peanut	55	29	70*	1	c
Hydrogenated cotton seed	67	26	69*	5*	a
Hydrogenated corn	58–80	34–18	64–70*	1–11*	a
Hydrogenated whale	83	—	—	—	j

Fats lowering serum cholesterol

Olive	79–90	7	85	8	a, c, j
Peanut	80–100	18	56	26	a, c, i
Rape seed	97–108	7	17	76†	j
Cotton seed	102–115	26	25	44	a, f
Sesame	103–118	12	45	43	e
Corn	103–128	9	35	56	a, b, g, h, j
Sunflower seed	125–140	10	30	60	c, f, h
Safflower seed	135–150	6	19	75	a, g, j
Soya-bean	129–143	14	29	57	i
Linseed	170–204	13	15	72	d
Whale	108–125	30	70		
Seal	130–170	18	82		c
Pilchard	160–183	26	74		c
Sardine	160–189	23	23	54	h

* Large proportion are *trans*-acids
† 51% is erucic acid

References: a. Ahrens, Hirsch, Insull, Tsaltas, Blomstrand & Peterson (1957); b. Beveridge, Connell & Mayer (1956); c. Bronte-Stewart, Antonis, Eales & Brock (1956); d. Eggstein & Schettler (1958); e. Gopalan & Ramanathan (1956); f. Gordon, Lewis, Eales & Brock (1957); g. Horlick & Craig (1957); h. Keys, Anderson & Grande (1957); i. Kinsell & Michaels (1955); j. Malmros & Wigand (1957)

has not yet been produced. This is hardly surprising, as a complete deficiency syndrome is produced only by rigid exclusion of these acids from the diet of the growing animal. In all animal species tested, they are essential for normal growth and health, and in the deficiency state wide-spread effects are seen. The facts that could be pertinent to the problem of atherosclerosis in man are:

i. Up to a certain age female animals require less of the poly-unsaturated fatty acids than males (Deuel, 1955).

ii. The requirement increases as the total fat intake increases (Deuel, 1955).

iii. The requirement may depend on the level of serum cholesterol, as they form a considerable proportion of the fatty acids esterified with cholesterol. The onset of the deficiency syndrome is accelerated by hypercholesterolaemia in the animal, whether it be from hypothyroidism, diabetes, the feeding of cholesterol or hydrogenated fat, presumably by draining of linoleate from the body-stores for esterification of cholesterol (Peifer & Holman, 1955). In the deficient state, deposition of cholesteryl esters is increased (Basnayake & Sinclair, 1956).

iv. Cell membrane permeability and capillary fragility are increased in the deficient state (Kramar & Levine, 1953), and maintenance of tissue structure appears to be one of their major biological roles.

Not all the poly-unsaturated fatty acids have biological activity, and those that have differ in degree. Linoleic and arachidonic acids appear to be the most potent; pyridoxine being necessary for the conversion of the former to the latter (Witten & Holman, 1952). Poly-unsaturated fatty acids with conjugated double bonds and *cis-trans* forms have no biological activity and, in fact, analogous to other situations where chemically related substances with differing physical structures behave as antimetabolites, *trans*-acids may be antagonistic to their natural *cis-cis* isomers (Holman & Aaes-Jørgensen, 1956). Not only does hydrogenation of an oil increase the proportion of saturated fatty acids but those poly-unsaturated acids that remain may lose their natural *cis-cis* configuration to become *trans*-acids. The degree to which this occurs depends on the hydrogenation technique used. *Trans*-isomers of C_{18} poly-unsaturated fatty acids are formed in the rumen (Lough & Garton, 1958) and therefore their presence in human dietary fats is not restricted to processed fats. The metabolic role of *trans*-acids in man, however, has yet to be studied.

3. Applications to Theories on the Pathogenesis of Ischaemic Heart Disease

In the triangular relationship between diet, serum cholesterol level, atherosclerosis and ischaemic heart disease, formulated from animal studies and epidemiological surveys in man, there is strong evidence for a direct association between diet and serum cholesterol trends in man. Far less convincing is the strength of the other links in this triple association. As a result, it is doubtful whether further application is justifiable at present. By its very nature the disease accounts for these difficulties. It is a disease of man's later years, and man's life-span is such that studies have to be relatively short-term, or use has to be made of the epidemiological approach where many variables operate concurrently. As yet, there is no precise measure of its prevalence or of its development in the living, and it will be seen, too, that there is no unanimity regarding its pathogenesis or the underlying pathology. For these reasons any policies regarding

preventive programmes that the alarming increase in incidence demands, would be founded more on assumptions than on facts. The basis for these assumptions demands closer examination.

a. Relationship of Coronary Atherosclerosis to Ischaemic Heart Disease

Ischaemic heart disease, the term accepted by the World Health Organization Study Group (1957), was defined as the cardiac disability, acute or chronic, arising from reduction or arrest of blood supply to the myocardium in association with disease processes in the coronary arterial system. It is generally accepted that atheroma forms the basis of this occlusive disease, but its relationship to myocardial infarction is not clear. Despite the rapid rise in mortality from ischaemic heart disease over the last few decades, Morris (1951) has claimed that the incidence of severe atherosclerosis has actually declined. The use of calcification and other criteria for the index of severity or acuteness of the pathological process may be open to question, but other data support this view. In the animal, atherosclerosis is easily produced but, apart from the recent findings of Donomae (personal communication, 1956) in Japan on feeding lanolin, the classical picture of myocardial infarction has not been reproduced so far by dietary means, although several investigators observed small fibrotic lesions in the myocardium of animals in which experimental atheroma had been induced (Heptinstall & Bronte-Stewart, 1954; Katz, Sanders, Megibow & Carlen, 1940; Leary, 1941; and others). It could not be assessed whether these changes resulted from a previous acute necrosis. The reported sex difference in the incidence of severe atherosclerosis found at autopsy is far less than the remarkable sex difference in incidence of myocardial infarction. It is, of course, well known that severe occlusive disease of the coronary arteries may exist without apparent ill effects, but the problem of relating the severity of the occlusive disease to its effects is made even more difficult when one notes that in a large proportion of cases with myocardial infarction at autopsy no subtending occlusion was found, despite careful dissection (Yater, Welsh, Stapleton & Clark, 1951; Branwood & Montgomery, 1956).

Indeed, it is conceivable that, in addition to atherosclerosis, a process may exist for the full explanation of the clinical manifestations. This has revived a great deal of interest in thrombotic and other mechanisms that interfere with circulatory flow, but the ischaemia could be initiated by a transient phenomenon or a primary block at capillary level, and the particular anatomical structure of the coronary circulation would favour retrograde stasis and thus account for the variable presence of the more acute occluding process in a major subtending branch. Ischaemic heart disease, then, may be the end-result of a series of processes, not least in importance being the state of the collateral circulation and the functional status of the heart at that time. It is not surprising therefore that the disease has protean manifestations, that the prevalence of the disease in the living is difficult to measure, and that there are certain difficulties in attaching much significance to minor differences reported from highly susceptible areas.

b. Pathogenesis of Atherosclerosis

One great obstacle to progress was overcome with the more precise definition of pathological terms which emphasized that atheroma or atherosclerosis is essentially a focal and predominantly an intimal change and should not be confused with generalized arteriosclerosis or other circulatory disorders. The essential components of the human atheromatous plaque appear to be intimal fibrosis and lipid infiltration. It is therefore not unnatural that most theories on its pathogenesis centre around either fat, fibrin, or both, but considerable controversy exists as to which component is primary and which secondary.

The filtration theory (Page, 1954) implies that the lipid infiltration is the primary process and that the fibrous tissue present indicates the reaction to the presence of fat. This theory postulates that centrifugal force of the circulation drives fat into contact with the arterial intima. Over the years, lipid deposition accumulates, but this is accelerated or intensified in the presence of blood lipid in excess amount or abnormal form, if the intraluminar or lateral filtration pressure is high or if the endothelium of the vessel walls is abnormally permeable. A satisfactory explanation therefore is at hand for the particular localization of the plaques at sites of pressure or velocity change within the circulatory system, and for the increased severity of atherosclerosis in the presence of hypertension and such disorders of lipid metabolism as diabetes mellitus and essential xanthomatosis. The steady encroachment of the plaque on the lumen of the vessel favours superadded thrombosis from circulatory stasis. In support of this theory, it is known that cholesterol is present in high concentrations in the lipid of the atheromatous plaque, the chemical nature of which resembles that of the blood plasma (Weinhouse & Hirsch, 1940). In-vitro studies have shown that cholesterol and lipid can penetrate the intima up to the internal elastic membrane, when serum is placed in contact with an artery (Wilens, 1951).

Apart from the finding of fat-soluble carotenoid pigment within the atheromatous plaque (Blankenhorn, Freiman & Knowles, 1956), there is no further indication that the fat in the plaque comes directly from the diet. Certainly the gross biochemical differences found in animal experiment and in population studies on groups with wide differences in disease prevalence would suggest that the blood lipids do exist in excessive amount and abnormal form. As atheroma develops in the animal and as the prevalence increases from group to group in man, the level of serum cholesterol increases and its distribution alters in that more and more is carried in the β-lipoprotein fraction, akin to reported differences between patients and controls (Barr, Russ & Eder, 1951; Barr, 1953; Oliver & Boyd, 1955). Similar differences accompany the lower susceptibility of the female below the age of 60 years, and after that age sex differences in both blood lipids and susceptibility disappear. An abnormal cholesterol–lipoprotein pattern is a frequent feature in diabetes mellitus, xanthomatosis, nephrosis and other diseases with a high incidence of ischaemic heart disease. Nevertheless, considerable overlap between affected and unaffected groups exists. In many recent studies within apparently homogeneous communities in highly susceptible areas, the differences seen are small, especially in the older age groups (Biörck, Blomqvist & Sievers, 1957). Although, as revealed by the Framingham study (Dawber, Moore & Mann, 1957), those with high levels of serum cholesterol are more liable to develop ischaemic heart disease than those with low values, the cholesterol–lipoprotein abnormalities at present recognized in ischaemic heart disease cannot be used with confidence to predict susceptibility in the individual.

c. Metabolic Effects of Alimentary Lipaemia Caused by Different Dietary Fats

Other biochemical abnormalities in ischaemic heart disease have led to various hypotheses regarding the development of the disease through a disturbance of fat metabolism. Barritt (1956) showed that the duration of postprandial lipaemia was more prolonged on the average in patients than in age-matched controls. Again, a marked overlap occurs and the differences are not significant until late in the lipaemic phase. As a result, it has been suggested that affected individuals lack a sufficiency of endogenous clearing factor, and certain experiments using heparin to activate the clearing mechanism have claimed proof of this (Oliver & Boyd, 1953). On the other hand, Moreton (1950) claimed that it was the biophysical state of dispersion of plasma lipids that was abnormal and that prolongation of the lipaemic phase would allow prolonged contact of abnormal lipids with the arterial intima and their subsequent deposition.

Different fats have different lipaemic responses. These differences in lipaemic response depend not only on the chain-length of the fatty acids but also on the degree of unsaturation of the long-chain fatty acids, in that oils with high iodine values produce an earlier peak and less prolonged lipaemia (Eggstein & Schettler, 1958). The lipaemic response to the same fat may alter after a period of dietary fat restriction (Pomeranze, Beinfield & Chessin, 1954) or merely after corn-oil supplements to the customary diet (Bronte-Stewart & Blackburn, 1958). Unfortunately, the significance of these effects is not clear, as the lipaemic response to the same fat in the same individual is at any rate very variable.

The difficulties Swank (1951) had in counting red cells in lipaemic blood led to cinematographic studies on the capillary circulation of the exposed cheek-pouch of the hamster (Swank & Cullen, 1953). Several hours after feeding cream they observed marked slowing with adhesiveness of the red cells, with concurrent changes in haematocrit values and erythrocyte sedimentation rate. About ten hours after the meal, the phenomenon passed off. Attempts to demonstrate this in man have, as yet, been unsuccessful, but it is of interest that the transient changes Bloch (1955) has observed in the capillary circulation soon after myocardial infarction are descriptively similar.

The electrophoretic rate of red cells in plasma decreases with age and is slower in patients with ischaemic heart disease (Davies, 1958). Washing the erythrocytes and suspending them in saline abolished these differences, while treating them with different fatty acids revealed that surface activity was proportional to the degree of unsaturation. Davies suggested that atheroma resulted from a reduction in the electrical charge borne by the individual lipid particles.

Other metabolic effects noted during alimentary lipaemia concern blood-clotting mechanisms. Merskey & Nossel (1957) could not show consistent differences in clotting times after feeding fats with different iodine values. The rate at which clots lyse, however, does appear to be affected thereby (Greig, 1956). Following an average breakfast of eggs, bacon and buttered toast, plasma fibrinolytic activity was markedly reduced. Removal of the chylomicron and β-lipoprotein fraction reversed this, and oils of high iodine value, when fed, enhanced fibrinolysis more often than they inhibited it (Greig & Runde, 1957). Another link with the cholesterol–lipoprotein system is the in-vivo demonstration by Kwaan & McFadzean (1957) that clots produced in the ear vein of cholesterol-fed rabbits lyse more slowly than in controls.

This approach is influenced by the thrombogenic hypothesis revived by Duguid (1954; Duguid & Robertson, 1957).[1] Here, the suggested initial lesion is a mural thrombus or fibrin encrustation on the arterial wall, which soon becomes organized and covered by endothelium. Vascularization occurs, and the fat present in the plaque could be the product of blood destruction from small haemorrhages recurring over long periods. Greig (1956) suggests that organization and endothelialization is an expression of failure on the part of the body to lyse the fibrin clot, and that the greater the intake of certain fats the more persistent would be the inhibition of fibrinolysis and the greater the incidence of severe atherosclerosis and thrombosis in the areas in which large amounts of animal fat are eaten.

Many other theories exist. Paterson (1954) has stressed the role of increased capillary fragility, and Kramar & Levine (1953) claim that increased capillary fragility can be reversed in man by changing the type of dietary fat and by feeding linoleate in the fat-deficient animal. Pyridoxine, too, necessary for the conversion of linoleate to arachidonate (Witten & Holman, 1952), has entered the field owing to the similarity to human atherosclerosis of the intimal proliferation and changes in ground substance seen in the arterial lesions of pyridoxine-deficient monkeys (Rinehart & Greenberg, 1951).

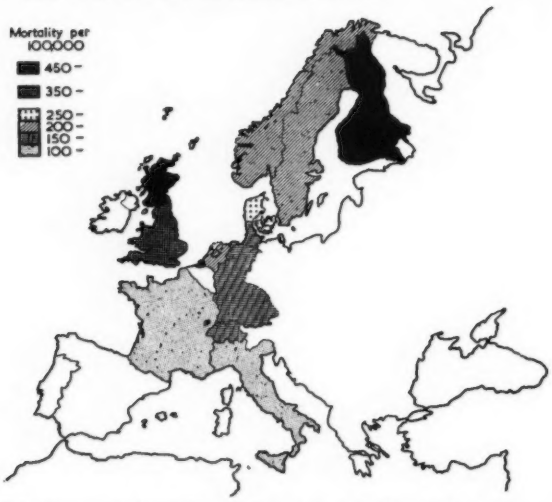
No matter which theory is propounded then, an explanation could be found in the nature of the dietary fat intake, especially its constituent fatty acids. In general, two broad trends emerge. Firstly, there is the long-continued excess of dietary fat, with its influence on the serum cholesterol–lipoprotein system. The latent interval before the onset of the disease, like that in the experimental animal, may be dependent on a time–dose relationship and influenced by certain local or general accessory factors. Secondly, there are the immediate effects of large fatty meals and sustained lipaemia on coagulation, fibrinolysis and circulatory flow. It may well be that both factors could be operative and both are in keeping with the epidemiology of the disease.

4. Applications to the Epidemiology of Ischaemic Heart Disease

Ischaemic heart disease is no problem amongst the fish-eating Japanese, maize-eating Bantu, and the olive-oil-eating peoples along the Mediterranean littoral. In these peoples, however, the total fat intake is low. It is in the areas where large amounts of fat are eaten that qualitative rather than quantitative differences in the dietary fat intake could explain better the reported differences in mortality from the disease. Scandinavian death-rates, with the exception of Finland (see fig. 6), are between one-third and one-half of those in the USA and Britain (World Health Organization, 1956), yet the total fat intakes are the same. The types of fatty foods contributing to these similar fat intakes are quite different. From the food balance-sheets of the Food and Agriculture Organization (1955) one may obtain an approximate estimate of this by computing the ratio of consumption of hard fats, viz., all animal and hardened vegetable fats, to liquid oil consumption which includes vegetable oils and the fat content of fish, cereals, nuts, etc. If one plots against the total fat intake for each country, firstly the corresponding death-rate and, secondly, this ratio (see fig. 7), a remarkable parallelism emerges. This parallelism is achieved only by the use of all

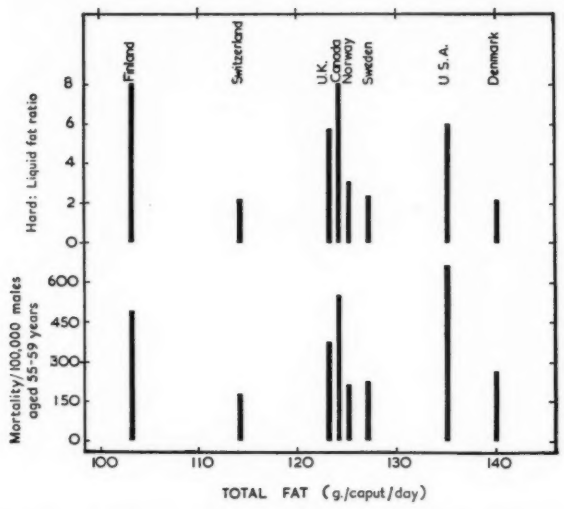
[1] See also Duguid, *Brit. med. Bull.* 1955, 11, 36.—Ed.

FIG. 6. GEOGRAPHICAL DIFFERENCES IN MORTALITY FROM ISCHAEMIC HEART DISEASE OF MALES AGED 55–59 IN WESTERN EUROPE



With minor variations, these differences are similar at all age ranges, with Scotland and Finland at the uppermost extreme and France and Italy at the lowermost. The mortality figures are somewhat higher in each case than as given with the key above. (Data from World Health Organization, 1956.)

FIG. 7. CORRELATION OF TOTAL FAT INTAKE AND OF THE RATIO HARD : LIQUID FAT IN DIETARY FAT WITH MORTALITY RATE FROM ISCHAEMIC HEART DISEASE IN DIFFERENT COUNTRIES



Qualitative differences in the available fat for consumption correlate with the different European death-rates from ischaemic heart disease better than quantitative differences. Canada and USA are included on this figure for comparison. (Dietary data on Finland supplied by Miss Hollingsworth; other data from Food and Agriculture Organization, 1955.)

three parameters. One could assume then that the liquid oils are protective at high fat intakes, and strength could be found for this assumption on the grounds that, in the animal, the essential fatty acid requirement increases as the total fat intake increases.

In Britain, the occupational mortality statistics could be related to the fact that the amount of dairy products and other animal fats eaten constitutes one of the most important social class differences in dietary habits of man (Ministry of Agriculture, Fisheries and Food, 1957), but the recent alarming increase in mortality from ischaemic heart disease is less easy to explain. Although the percentage of calories from all fats has risen by about 10% to over 40% during the last 25 years in the USA (Jolliffe, 1957), no such remarkable rise has occurred in Britain. A considerable qualitative change has occurred, however, with a rise in consumption of fats containing saturated fatty acids at the expense of the poly-unsaturated fatty acids. For example, there has been a decreased demand for fish and whole-grain cereal because of the increased use of processed germ-free breakfast cereals and highly refined flours, but the most obvious change is the striking rise in consumption of margarine and other hydrogenated products (Sinclair, 1956; Ministry of Agriculture, Fisheries and Food, 1957). Other changes have occurred—many dependent on current developments in animal husbandry. For example, it is very possible that eggs eaten a generation ago were quite different from those of today because the proportion of linoleate therein decreases sharply as the hen's diet is changed from whole-grain cereal to one poor in linoleate content (Reiser, 1951). Butter-fat from stall-fed animals has a lower iodine value than that from animals grazing off summer pasture (Bailey, 1945). Not only does prime quality beef contain a greater content of interstitial fat, but the iodine value of beef-muscle fat is much lower than that of subcutaneous fat. Many of these changes are subtle and, when taken selectively (Yudkin, 1957), are less convincing than the sum-total change.

Of all the epidemiological features, it is to the remarkable sex difference that the hypothesis of dietary fatty acid imbalance is best applied. When one notes that at a given age, the American female dies more often from the disease than the Japanese male, and that the recent rise in death-rate applies to both male and female, a factor in addition to the sex hormones cannot be denied. The sex difference is remarkable only in the highly susceptible areas where a great deal of fat is eaten (Walker, Andersson & Bersohn, 1956; Keys, 1956b), and the possibility exists that the female has a greater metabolic capacity for handling fat. Although there is no evidence for this in human beings, it is known that in the animal, up to a certain age, the requirement for essential fatty acids is far less in the female (Deuel, 1955).

Undeniable proof of a relationship of an imbalanced fatty acid intake to these epidemiological trends would be difficult to obtain. Most dietary surveys need cautious interpretation and, of all food items, fats and oils, with the unknown wastage in the kitchen, are the least accurate to measure. Even less accurate would be the use of food tables to compute the fatty acid composition, as the iodine value of a fat fluctuates widely and depends on where and when the plant was grown and how the animal was fed. Account, too, may have to be taken of the presence of accompanying tocopherols and other antioxidants, and allowance made for different methods of margarine manufacture owing to the possible anti-metabolite action of *trans*-acids.

Despite these difficulties, Rose (personal communication, 1958) was able to show differences in the type of fatty acids consumed by subjects with atherosclerosis as against matched controls from the same area in London. Only two long-term trials (Morrison, 1955; Lyon, Yankley, Gofman & Strisower, 1956) are available to assess the therapeutic effect of dietary fat restriction in ischaemic heart disease. Neither has made allowance for the possibly beneficial role of the poly-unsaturated fatty acids and neither could claim to fall within the category of a controlled clinical trial. In both, a markedly increased number survived in the group where the dietary fat intake was sharply reduced.

5. Conclusion

The purpose of this paper is to examine how closely the various features and hypotheses concerning ischaemic heart disease could be related to the dietary fat intake. It is concluded that, although the level of serum cholesterol and the nature of the dietary fat intake are closely related, the hypothesis that ischaemic heart disease results from a disturbance in fat metabolism is founded more on assumption than on fact. Undoubtedly other factors are important in its aetiology, and a possible interplay of genetic and environmental factors has not been excluded. Nevertheless, of all the environmental variables constituting our present-day mode of life, no other at present known can be so closely related to pathological, metabolic and epidemiological features of the disease as the nature of the dietary fat intake. It should be emphasized again that the evidence for such a relationship is purely circumstantial and that the hypothesis is applied to a disease both difficult to measure in its prevalence and to predict in its mode of progression. The implications are wide and affect developments in animal husbandry and methods of processing food, and principles regarding food values and food preferences should be stressed in the education of our youth of today. For these reasons alone, a great deal more factual evidence is desirable and, of all the environmental factors, dietary fat intake is the one most susceptible to measure and control. The present-day dietary fat intake therefore stands out as a challenge for further testing in order to define its precise role in the alarming increase in death-rate from ischaemic heart disease. Certainly few problems merit closer attention at the present time.

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FATS AND BLOOD COAGULATION

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1 Effects of fatty meals on blood coagulation in man
  a Effects of fatty meals on the clotting time of plasma in the presence of Russell's viper venom
  b Effects of fatty meals on the clotting time of whole blood in silicone-coated tubes
  c Effect of fatty meals on other clotting tests
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  a Early work on "cephalin"
  b Coagulant activity of chylomicra and of crude lecithin
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During the past few years evidence has accumulated which suggests that eating fat in excessive amounts may be an aetiological factor in atherosclerosis and thrombosis. This evidence has been adequately reviewed by several authors (e.g. Fullerton, Davie & Anastasopoulos, 1953; Poole, 1954; O'Brien, 1957c) and will not be restated here. If excessive ingestion of fat is harmful, the mechanism of the harmful effects is clearly of great practical importance, and a number of theories about the mechanism have been put forward. The suggestion that increased coagulability of the blood after fatty meals might be a factor in the development of thrombosis was first clearly made by Fullerton *et al.* (1953). These authors reported the effects of fatty meals given to human subjects on the clotting time of whole blood in silicone-coated tubes and on the clotting time of plasma in the presence of Russell's viper venom. In both cases they observed that a fatty meal led to a shortening of the clotting time. These observations and their possible practical importance led to extensive studies of the participation of various fatty substances in blood coagulation. None of this work has contributed much to deciding whether or not the changes in coagulability following fatty meals are implicated in the pathogenesis of thrombosis. However, this work has provided information about the chemical mechanisms which underlie blood coagulation. In this paper the experimental facts which have emerged will be described; speculation about their practical significance seems unprofitable at this stage. In particular, it is quite unwarrantable to offer to the general public any advice on what they should or should not eat in order to avoid thrombosis (Pincher, 1956).

The role of fats in blood coagulation has been studied in two principal ways: (i) by giving fatty meals to human subjects and carrying out various clotting tests before and after the meal; (ii) by adding various fatty substances to plasma in vitro. These two approaches to the problem will be considered separately.

1. Effects of Fatty Meals on Blood Coagulation in Man

Many studies of the effects of ingestion of fat on clotting in man have been published during the last ten years. The results at first sight appear confusing. The confusion is partly due to the fact that different workers have used different clotting tests. Some workers have expressed decided views about the relative value of different clotting tests as indicators of changes of pathological interest.[1] Until more is known about the relationship between clotting and thrombosis there can be no grounds for believing that one clotting test is a better indicator of pathological change than another.

a. Effects of Fatty Meals on the Clotting Time of Plasma in the Presence of Russell's Viper Venom

Fullerton & Anastasopoulos (1949) showed that fatty meals given to human subjects caused a definite and consistent shortening of the clotting time of plasma in the presence of Russell's viper venom. This finding was the physiological counterpart of some studies in vitro by Macfarlane, Trevan & Attwood (1941) which are discussed below. The observations of Fullerton and Anastasopoulos were confirmed by Fullerton *et al.* (1953), O'Brien (1955), Maclagan & Billimoria (1956), Hall (1956) and Merskey & Nossel (1957).

Hall pointed out that the shortened clotting time after eating fatty food was demonstrable only if the platelets had been removed from the plasma by centrifuging; he was unable to demonstrate a change in the presence of platelets. At first sight this finding suggests that the shortening in clotting time which can be shown in the absence of platelets is not relevant to any pathological processes which may occur in vivo, since the circulating blood undoubtedly contains platelets. Such a conclusion, however, may not be justified. O'Brien (1958) showed that intact platelets had no effect on plasma coagulation in the presence of Russell's viper venom but that, when the platelets were broken up in a mechanical disintegrator, a potent accelerator of clotting was released. It is not known at present whether or not platelets undergo any change in the body analogous to mechanical disintegration.

Different types of fat eaten in equivalent amounts produce different degrees of shortening of the clotting time of plasma in the presence of Russell's viper venom. O'Brien (1956b) showed that eggs produced a more profound effect than did butter, margarine or a proprietary vegetable cooking fat.

b. Effects of Fatty Meals on the Clotting Time of Whole Blood in Silicone-Coated Tubes

Duncan & Waldron (1949) and Waldron, Beidelman & Duncan (1951a, 1951b) showed that there was a shortening of the clotting time of whole blood in silicone-coated tubes after a fatty meal, but this was denied by Tulloch, Overman & Wright (1953). If, however, the experimental findings of Tulloch *et al.* are analysed by statistical methods, it is seen that nine out of ten of their subjects did in fact show a significant shortening.

Fullerton *et al.* (1953) and O'Brien (1955) found a shortening of the clotting time of whole blood in silicone-coated tubes,

[1] For an assessment of tests of clotting function see R. Biggs, *Brit. med. Bull.* 1955, 11, 5.—Ed.

but Manning & Walford (1954) did not. Merskey (1956) and Merskey & Nossel (1957) found a shortening in the time with one type of meal but not with another. These authors contended that shortening which occurred in their experiments might not be significant, although statistical analysis suggests that it is. In any case, if there is a shortening of the whole-blood clotting time in silicone-coated tubes after ingestion of fat, the change is not a large one.

c. Effect of Fatty Meals on Other Clotting Tests

Vandenbroucke, Lauwers & Verstraete (1954), O'Brien (1955) and Pilkington (1957a) all reported that fatty meals in man caused a shortening of the calcium time (i.e., the clotting time of citrated plasma after the addition of calcium). Merskey & Nossel (1957) did not confirm this finding. O'Brien (1955) found a change in thrombin generation from plasma; this again was not confirmed by Merskey & Nossel (1957). Merskey and Nossel used several other clotting tests which revealed no change in their experiments.

The available information about changes in blood coagulability in man after fatty meals may be summarized as follows: in the presence of Russell's viper venom there is a definite shortening of the clotting time of plasma from which most of the cellular elements have been removed by centrifugation; no other clotting test has demonstrated any unequivocal change.

2. Effects on Coagulation of Adding Fatty Substances to Plasma in Vitro

a. Early Work on "Cephalin"

Recent studies have been particularly concerned with the participation of phosphatides in coagulation, and it is necessary to point out that the idea that these substances are important in this respect is not new. A great deal of work has been done with the supposed compound "cephalin". Advances in the chemistry of phosphatides have now shown that "cephalin" is a mixture of substances, and the chemical composition of the preparations used by earlier workers cannot now be determined. For a long time it was believed that the acetone-insoluble, ether-soluble fraction of brain tissue comprised two substances: lecithin, which was soluble in ethanol, and cephalin, which was not. The chemical structure of lecithin was correctly worked out, and it was thought that cephalin was what we now know as ethanolamine phosphatide or phosphatidylethanolamine. However, in 1930 Rudy & Page showed that the classical "lecithin" fraction was really a mixture of lecithin and "cephalin" (i.e., ethanolamine phosphatide). In 1942 Folch showed that the classical "cephalin" fraction was a mixture of at least three substances: ethanolamine phosphatide, serine phosphatide and a phosphatide containing inositol. This means that earlier studies with either "lecithin" or "cephalin" were carried out with complex mixtures. The belief that ethanolamine phosphatide was insoluble in ethanol led to the erroneous idea that it could be purified by repeated extraction with ethanol. Some preparations supposed by earlier workers to be pure ethanolamine phosphatide probably contained none.

b. Coagulant Activity of Chylomicra and of Crude Lecithin

Macfarlane & Barnett (1934) studied the coagulant properties of various snake venoms and concluded that the venom of *Vipera russelli* was the most active. In the course of studying the properties of Russell's viper venom, Trevan & Macfarlane (1936) found that the clotting time of plasma in the presence of Russell's viper venom was shortened when an aqueous emulsion of a commercial preparation of lecithin was added. This finding was confirmed by Leathes & Mellanby (1939), by Witts & Hobson (1940) and many other workers. Macfarlane *et al.* (1941) then showed that, when plasma was freed from particulate fat by high-speed centrifugation, its clotting time in the presence of Russell's viper venom was prolonged, but could be restored to normal if the fatty supernatant material removed by centrifugation was put back or if crude lecithin or cream from the top of a bottle of milk was added. It therefore seemed likely that the clotting time of plasma in the presence of Russell's viper venom was controlled by, among other things, the concentration of a substance present in crude lecithin, in chylomicra and in milk-fat.

These observations were not pursued for some time, since it was not clear that the substances involved had any effect on clotting unless Russell's viper venom was present. The work of Fullerton *et al.* (1953) described above stimulated a search for techniques not involving Russell's viper venom by which the activity of chylomicra or crude lecithin in vitro could be demonstrated. Poole (1955a) showed that when plasma was freed from chylomicra by centrifuging at 23,000 *g* for one hour its clotting time was about twice that of plasma centrifuged at 2,500 *g* for 20 minutes (which is sufficient to remove all visible cellular material). The clotting time of the plasma spun at high speed could be brought down to the control level by the addition of a washed suspension of the chylomicra removed by centrifuging, of crude lecithin, or of a washed suspension of chylomicra obtained from the thoracic duct of a rat fed olive-oil. Essentially similar results were later described by Kingsbury & Morgan (1957).

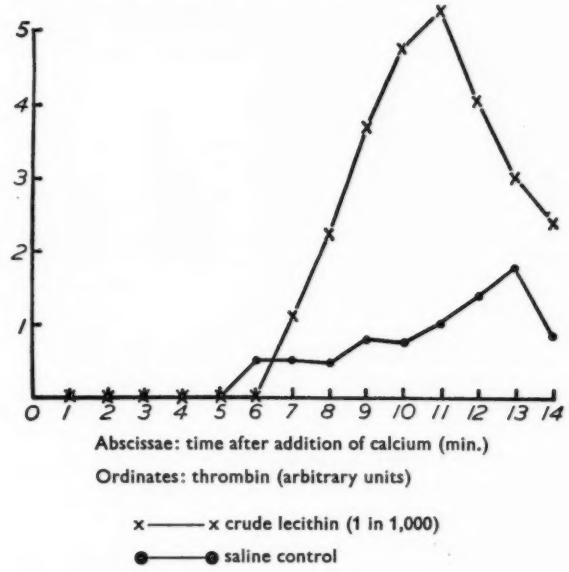
O'Brien (unpublished observations, 1955; confirmed by Poole, 1957) made an important observation about the activity of crude lecithin. Using plasma from which the cells but not the chylomicra had been removed by centrifuging, O'Brien showed that, while adding crude lecithin did not shorten the clotting time, it did greatly increase the amount of thrombin formed in the clotting process. O'Brien's technique was that described by Pitney & Dacie (1953), which is a modification of the test introduced by Macfarlane & Biggs (1953). The results of one such experiment are shown in fig. 1. In this test system, adding a suspension of washed chylomicra to plasma produces the same result as adding crude lecithin.

c. Ethanolamine Phosphatide as the Active Coagulant Substance in Chylomicra

As a result of these observations, investigations were made to discover the nature of the chemical substance or substances responsible for the activity of chylomicra. Robinson (1955) had shown that chylomicra obtained from the thoracic duct of a rat fed olive-oil consisted of triglycerides and small amounts of cholesterol and phosphatides. Most of the phosphatide fraction consisted of lecithin which apparently formed a surface layer around the chylomicra. The active substance was present in ethanol–ether extracts of chylomicra and was heat-stable. It seemed likely, therefore, that the coagulant activity was due to one or more lipoid substances. Since triglycerides and cholesterol were found to be inactive, it appeared probable that the activity resided in the phosphatide fraction of the chylomicra.

At this stage of the investigation it looked very much as if

FIG. 1. THE EFFECT OF CRUDE LECITHIN ON THE GENERATION OF THROMBIN FROM "RECALCIFIED" CITRATED PLASMA


5
4
3
2
1
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Abscissae: time after addition of calcium (min.)
Ordinates: thrombin (arbitrary units)
x——x crude lecithin (1 in 1,000)
●——● saline control

the active substance was lecithin. Commercial preparations of lecithin, however impure they might be, presumably contained some lecithin; and it was known that lecithin occurred in the chylomicron. However, when experiments were carried out with samples of purified lecithin, it was found that they were completely inactive (Poole, Robinson & Macfarlane, 1955). It was observed that lecithin solutions which had been left on the bench for two weeks developed slight activity (this was subsequently confirmed by Bidwell & Biggs, 1957), but, even so, this activity was not nearly as great as that of crude lecithin preparations or of chylomicra. It was clear, therefore, that the coagulant activity of chylomicra could not be ascribed to lecithin, but the arguments for supposing that the active substance was a phosphatide still held good.

Tests were then carried out to see if any of the other known phosphatides possessed activity resembling that of chylomicra. Purified samples of ethanolamine phosphatide, serine phosphatide and the inositol-containing phosphatide from brain tissue were tested. It was found that ethanolamine phosphatide was strongly active, whereas the other two showed no activity in the test systems used: these were the acceleration of the clotting time of plasma in the presence of Russell's viper venom (Poole & Robinson, 1956a) and the thrombin generation test (Robinson & Poole, 1956a, 1956b) (see fig. 2).

Before the coagulant activity of chylomicra could be attributed to ethanolamine phosphatide, it was necessary to show that this substance was present in chylomicra and that it occurred in a concentration that could account for the activity of the chylomicra. Chylomicra, on acid hydrolysis, yielded ethanolamine (Robinson & Poole, 1956b) and, on mild alkaline hydrolysis, glycerylphosphorylethanolamine, in approximately equivalent amounts (Dawson, 1956, unpublished observations). The phosphatide fraction of chylomicra appeared to consist of approximately 90% lecithin, 5% serine phosphatide and 5% ethanolamine phosphatide. Good agreement was found between the coagulant activity of suspensions of chylomicra and of solutions of ethanolamine phosphatide of approximately equivalent concentration.

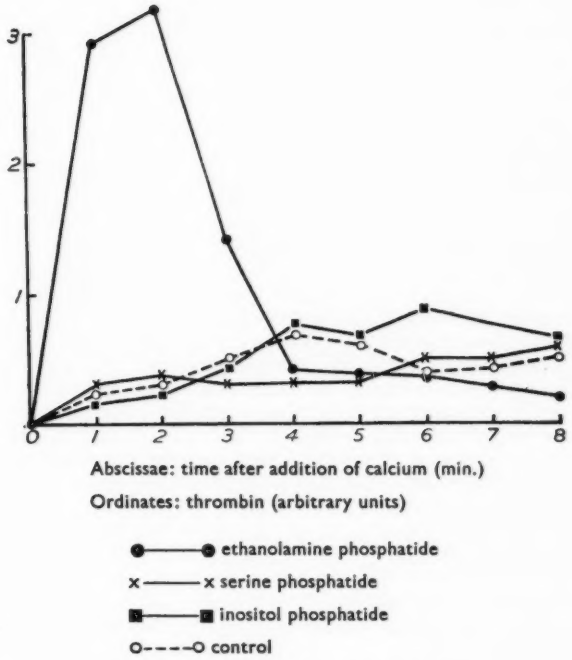
Further information about the coagulant activity of ethanolamine phosphatide was provided by Poole & Robinson (1956b). It was shown that ethanolamine phosphatide from eggs was as active as that from brain; so were two samples of synthetic dimyristoyl ethanolamine phosphatide; a sample of synthetic dipalmitoyl ethanolamine phosphatide was also active but rather less so, perhaps because it proved impossible to disperse it in water as thoroughly as the other samples (figs. 3, 4). It was also shown that in a series of assorted lipoid fractions of brain tissue the coagulant activity per unit weight was correlated with the ethanolamine content of the acid hydrolysate.

That substances rich in ethanolamine phosphatide show coagulant activity of the type described has been confirmed by O'Brien (1956a), Maclagan & Billimoria (1956), Garrett (1956) and Barkhan, Newlands & Wild (1956). Not all these workers used the same clotting tests, but it is probable that they were all demonstrating the same type of activity.

d. Possible Effects of Other Phosphatides on Blood Coagulation

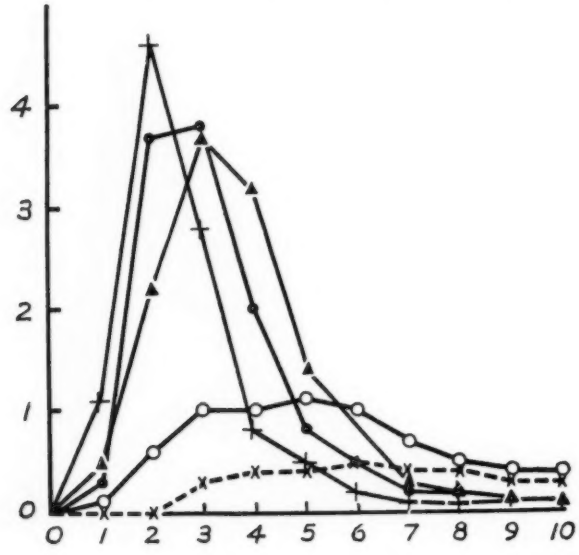
The results described show that ethanolamine phosphatide has an effect on coagulation and that lecithin has not. The

FIG. 2. THE EFFECTS OF ETHANOLAMINE PHOSPHATIDE, SERINE PHOSPHATIDE AND INOSITOL PHOSPHATIDE FROM BRAIN TISSUE ON THE GENERATION OF THROMBIN FROM "RECALCIFIED" CITRATED PLASMA



One mg. of phosphatide was added to each ml. of plasma.

FIG. 3. THE EFFECTS OF VARIOUS PURIFIED AND SYNTHETIC SAMPLES OF ETHANOLAMINE PHOSPHATIDE ON THE GENERATION OF THROMBIN FROM "RECALCIFIED" CITRATED PLASMA



Abscissae: time after addition of calcium (min.)

Ordinates: thrombin (arbitrary units)

●——● ethanolamine phosphatide from brain

▲——▲ synthetic DL-α-dimyristoyl ethanolamine phosphatide

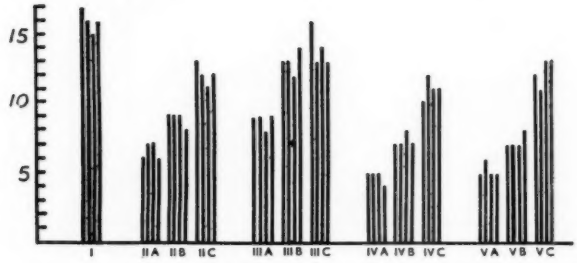
+——+ synthetic L-α-dimyristoyl ethanolamine phosphatide

o——o synthetic DL-α-dipalmitoyl ethanolamine phosphatide

x- - - -x distilled water control

One mg. of phosphatide was added to each ml. of plasma.

FIG. 4. THE EFFECTS OF VARIOUS PURIFIED AND SYNTHETIC PHOSPHATIDES ON PLASMA COAGULATION IN THE PRESENCE OF RUSSELL'S VIPER VENOM



Ordinates: clotting time (sec.)

Four replicate determinations of the coagulation time were made for each substance at each concentration.

I: distilled water control

II: synthetic DL-α-dimyristoyl ethanolamine phosphatide

III: synthetic DL-α-dipalmitoyl ethanolamine phosphatide

IV: synthetic L-α-dimyristoyl ethanolamine phosphatide

V: egg ethanolamine phosphatide

A: 1 mg./ml.; B: 100 μg./ml.; C: 10 μg./ml. All concentrations are added concentrations.

position regarding other phosphatides is at present not clear. Both Barkhan *et al.* (1956) and O'Brien (1957b) stated that serine phosphatide at low concentrations slightly accelerated clotting in the presence of Russell's viper venom but was inhibitory at higher concentrations. On the other hand, Poole & Robinson (1956a), Garrett (1956) and Biggs & Bidwell (1957) demonstrated no activity with samples of serine phosphatide. Barkhan *et al.* (1956) found a maximal accelerating activity at 0.04 mg./ml. and an inhibitory action above 0.5 mg./ml. O'Brien's sample, however, showed maximal acceleration at 1 mg./ml. and inhibition at 100 mg./ml. These gross differences in the effects of different concentrations raise the question whether the coagulant activity reported might not be due to trace contaminants present in different amounts in these two samples and absent from those tested by other workers. O'Brien (1957b) found activity with inositol-containing phosphatide fractions, a finding which is also at variance with previous reports.

Other purified and synthetic phosphatides which have been tested and found inactive by various workers now include lysoethanolamine phosphatide, ox-brain acetal, phosphorylcholine, phosphorylserine and monostearoyl lecithin (Poole & Robinson, 1956b; Biggs & Bidwell, 1957).

e. Ethanolamine Phosphatide in Platelets

Since both platelets and ethanolamine phosphatide, when added to plasma freed from platelets by centrifugation, increase the amount of thrombin demonstrable in the thrombin generation test, it seemed possible that the activity of platelets might be at least partly due to ethanolamine phosphatide (O'Brien, 1956a). Robinson (1956, unpublished observations) showed that acid hydrolysates of lipoid extracts of platelets contained ethanolamine. Wallach, Surgenor & Walters (1956) have also produced evidence that platelets contain ethanolamine phosphatide.

f. Ethanolamine Phosphatide as an Essential Substance in Blood Coagulation

The belief that the coagulant activity of chylomicra was due to ethanolamine phosphatide led to an investigation of whether this substance was essential for normal coagulation or whether it merely enhanced the normal mechanism. Since it was already known that the clotting time of plasma could be extended beyond the normal range by high-speed centrifugation (Poole, 1955a), and since it was believed that this extension was due to removal of ethanolamine phosphatide, the effect of even more rapid centrifugation was tried. The clotting time of plasma was extended to 70 minutes by this means and then restored to the control level of three minutes by adding ethanolamine phosphatide (Poole, 1957). Subsequently Robinson, Harris & Poole (1957a, 1957b) found that the clotting time of plasma could be prolonged still further if the plasma was incubated with a phosphatidase produced

by a strain of *Bacillus cereus*. The enzyme preparation was similar to that of Chu (1949). Again the clotting time could be brought back to normal by adding ethanolamine phosphatide.

These results are consistent with the hypothesis that ethanolamine phosphatide is an essential substance for blood coagulation.

g. Fatty Acids and Blood Coagulation

The effects of adding free fatty acids to plasma have been studied by Poole (1955b), Pilkington (1957b) and O'Brien (1957b). Certain fatty acids shorten the clotting time of plasma either with or without Russell's viper venom. The results of the two tests do not, however, run parallel. The calcium time is shortened by saturated fatty acids with a chain-length of 16 carbon atoms or more, the effect increasing with increasing chain-length up to 20 carbon atoms. There is at present no information about fatty acids with longer chains. With Russell's viper venom, however, lauric acid (C_{12}), which has no effect on the calcium time, is more active than stearic acid (C_{18}). Stearic acid has an effect on the calcium time in very low concentrations. Robinson, Harris, Poole & Jeffries (1955) showed that in man eating fat leads to a rise in the concentration of free fatty acids in the plasma; this concentration is within the range over which an effect can be demonstrated on clotting in vitro. It is therefore possible that free fatty acids contribute to the coagulation changes which follow a fatty meal.

3. Coagulation and Thrombosis

The Greek word θρόμβος means a clot (in the most general sense). By usage, however, blood clotting has come to mean the solidification of blood outside the body, whereas thrombosis refers to the deposition of solid material from the blood on to the inner wall of a blood vessel. It is still too often assumed that factors which influence clotting must necessarily influence thrombosis and conversely that factors which do not affect clotting cannot be concerned in thrombus formation. Such assumptions are not justified. A thrombus is morphologically different from a clot, and it is difficult to believe that changes in the vessel wall, particularly in the endothelium, do not contribute to thrombosis. Since the work of Hewson (1772), who showed that blood does not clot in isolated segments of undamaged veins, it has been clear that vascular endothelium differs markedly from glass test-tubes in its effect on blood. Removal of endothelium does not, however, necessarily lead to thrombosis (Poole, Sanders & Florey, 1958). Later work may well show that factors which are important in coagulation are unimportant in thrombosis or vice versa. The comparison by Jewell, Pilkington & Robinson (1954) of the effects of different anticoagulants on experimental thrombosis of rabbits' ear veins suggests that there are important differences between the prevention of clotting and the prevention of thrombosis. The work of Kwaan, Lo & McFadzean (1957a, 1957b) emphasizes that different experimental techniques for producing thrombosis may produce lesions which behave differently. The work of these same authors, together with that of Greig (1956) and Greig & Runde (1957), calls attention to the possibility that alterations in the fibrinolytic mechanism produced by ingestion of fat might play a part in determining the fate of thrombi.

In short, it appears that not enough is known about the connexion between clotting and thrombosis to decide whether or not the part played by fats in blood coagulation is relevant to the mechanism of thrombosis. If further studies show that there is such a connexion, then the results described above may be of practical importance. If not, then, to paraphrase the remarks of O'Brien (1957a), the only practical conclusion which can be drawn from this work is that people who have recently consumed a large fatty meal should be particularly careful to avoid being bitten by snakes.

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ESSENTIAL FATTY ACIDS AND THE SKIN

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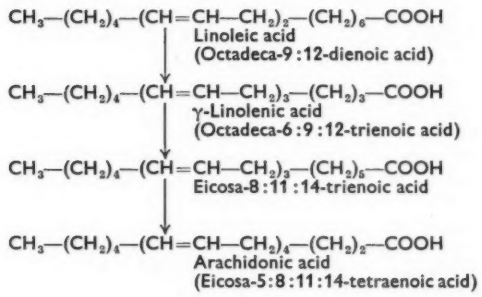
1 Nature of essential fatty acids
2 Macroscopical appearance of the skin in deficiency of essential fatty acids
3 Histological appearance of the skin in deficiency of essential fatty acids
4 Follicular hyperkeratosis in man
5 Eczema in infants
6 Structural function of essential fatty acids
7 Permeability of the skin in deficiency of essential fatty acids
References

1. Nature of Essential Fatty Acids

An essential fatty acid is defined as a fatty acid that cannot be synthesized by the body in sufficient amount and must therefore be provided in relatively small amount unless a closely related substance is available to the body, since it or a closely related substance is essential for the bodily processes of metabolism, growth or repair. In giving this definition some years ago (Sinclair, 1952), I pointed out that the synthesis of some linoleic and arachidonic acids in the body did not prevent them from being regarded as essential nutrients: the relevant point is whether sufficient can be synthesized for the needs of the body under given conditions. There are several functions of essential fatty acids (EFA). For some, arachidonic acid is probably absolutely required; for others, either linoleic or arachidonic acid may serve; for yet others, it may well be that a variety of polyenoic acids are effective though in differing degree. In some cases, though not in all, this is because linoleic acid can be converted in the body into arachidonic.

The conversion of linoleic acid into arachidonic has been studied in the laboratories of Mead in the United States of America (University of California Medical School, Los Angeles) and in those of Klenk in Cologne, Germany. By the use of ^{14}C it was shown that this conversion involves the lengthening of the carbon-chain of linoleic acid at the carboxyl-end by the addition of an acetate unit (Mead, Steinberg & Howton, 1953; Klenk, 1954, 1956). Mead & Howton (1958) suggested that this conversion involves γ-linolenic (octadeca-6:9:12-trienoic) acid and a C_{20}-trienoic acid as intermediates. These steps are illustrated in the accompanying scheme.



It is alleged (Witten & Holman, 1952) that pyridoxin (vitamin B_6) is necessary for this transformation. If this is so, deficiency of arachidonic acid, which appears to be the most important EFA, could occur from dietary deficiency of linoleic acid or from dietary deficiency of pyridoxin; arachidonic acid itself is seldom found in food-stuffs and does not occur in any plant source.

2. Macroscopical Appearance of the Skin in Deficiency of Essential Fatty Acids

Following the demonstration by Evans & Burr (1927) of the need for fat in the diet of the rat, Burr & Burr (1929) characterized the syndrome as consisting of certain skin lesions, renal lesions and failure of growth. They concluded (Burr & Burr, 1930) that ". . . linoleic acid is the essential unsaturated fatty acid, with the possibility that arachidic acid [*sic*] may also be involved"; they added that the rats deficient in EFA drank twice as much water as the controls. Severe skin lesions have also been described as a prominent sign of fat-deficiency in the mouse (White, Foy & Cerecedo, 1943), dog (Hansen & Wiese, 1951), guinea-pig (Reid, 1954), calf

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(Lambert, Jacobson, Allen & Zaletal, 1954) and pig (Witz & Beeson, 1951).

Scaliness of the skin, or dandruff, is the main macroscopical lesion that has been observed in lower animals. This sign is non-specific, being found in many nutritional and non-nutritional dermatoses. Even in EFA-deficiency it is not invariable: Evans & Lepkovsky (1932) omitted it from their list of the "essential facts" of fat-deficiency, although they had seen the sign "almost always" in their EFA-deficient rats. Brown & Burr (1936) showed that there was an inverse relationship between scaliness and humidity of the atmosphere; Aaes-Jørgensen & Dam (1954a, 1954b) noted that scaliness was absent in an atmosphere of relative humidity of 80–90% but was present at 45–50%. This might be just the general observation that nutritional dermatoses are usually intensified by dry atmospheres and decreased by humid ones (see Collins, Schreiber & Elvehjem, 1953), but in the case of EFA-deficiency there is the particular reason of permeability of the epidermis to water which will be discussed presently.

The Burrs referred to "scaly tail" in their descriptions of EFA-deficiency in the rat but this is a bad term because the rodent's tail is normally beset with rows of scales—as is known to zoologists who consider that mammals originated from reptilian ancestors. The ridged or annulated tail has been observed by various workers (Hume, Nunn, Smedley-Maclean & Smith, 1938; Quackenbush, Steenbock, Kummerow & Platz, 1942; Barki, Nath, Hart & Elvehjem, 1947; Panos & Finerty, 1953).

"Caudal necrosis" was also recorded by Burr & Burr (1929), by which was meant dry gangrene of the distal part of the tail. Its occurrence has been confirmed by McAmis, Anderson & Mendel (1929) (in one of their rats), Barki *et al.* (1947) in the mature rat, and White *et al.* (1943) in the mouse. Other workers have stated that they have not met with the sign in their experience (Evans & Lepkovsky, 1932; Sinclair, 1952; Kramár & Levine, 1953).

Certain other macroscopical changes occur in the skin. Infections occur very readily; mucocutaneous junctions show ulceration and fissuring; petechiae may occur. In the skin of the normal rat superficial brown squames are found on the dorsum of the back after the animal is about six weeks old. A similar brown colour develops along the free edges of the tail scales. This brown coloration occurs first at the mouths of the hair follicles and is probably connected with sebum. In EFA-deficiency this brown epidermis does not develop, which may indicate an abnormal quality of sebum.

In the hooded rat a striking sign is browning of the black hood hair, apparent after about 20 weeks on a diet deficient in EFA. Since small tracts of new jet-black hair are found to grow very slowly against the general brown background, there is no intrinsic local inability to form the usual black pigment but merely a slowing of the process. After therapy with EFA a very prominent sign is the rapid growth of jet-black hair replacing the brown. In EFA-deficiency hair replacement is poor (Rokonnes, 1953). The brown hairs may therefore owe their colour to fading of the original black pigment.

3. Histological Appearance of the Skin in Deficiency of Essential Fatty Acids

Various authors have described the histological appearance of the skin in the rat (Ramalingaswami & Sinclair, 1953) and in the dog (Hansen, Holmes & Wiese, 1951; Hansen, Sinclair & Wiese, 1954). In the rat deficient in EFA the epidermis is increased in thickness two to three times, this increase occurring both in the stratum malpighii and granulosum. The latter layer is composed of three or four layers of cells instead of the normal single flattened layer, and its cells are packed with numerous irregular densely-staining granules. The stratum corneum is also markedly increased in thickness, the keratin layers being closely packed and adherent. This hyperplasia of the surface epithelium increases in severity in the region of the openings of hair follicles, and the openings of the follicles become blocked. This acanthosis and hyperkeratosis of the epithelial lining of the follicular openings extends only for a short distance down the main body of the hair follicle and does not reach down as far as the opening of the sebaceous gland into the follicle. The sebaceous glands themselves are hypertrophied, and in male animals contain a fine granular basophilic material.

In the corium the subcutaneous fat is not markedly decreased. In the superficial part of the corium, immediately underneath the basal layer of the epidermis, a marked increase and distension of the capillaries and a mild mononuclear cellular infiltration occur in places, not specifically in the regions of the follicular openings. The mesenchymal ground substance is defective and there is fragmentation of collagen; in the ear the cartilage is thrown into folds and grossly distorted.

4. Follicular Hyperkeratosis in Man

Follicular hyperkeratosis occurs in many conditions, such as deficiency of calories, of EFA, vitamin A and (in a different type) of ascorbic acid. The commonest form is that found on extensor surfaces of the limbs in well-nourished persons, particularly girls around puberty who are slightly obese. This common lesion is much more frequent in the obese and is quite different from the follicular hyperkeratosis of deficiency of vitamin A in that the skin in the former is not dry. In deficiency of calories, as in starvation, there is likely to be deficiency of EFA since, although raw vegetable soups are the usual food in times of severe caloric deficiency, the unsaturated fatty acids these contain are easily oxidized and will be used as fuel in the body. Further, the common form of follicular hyperkeratosis in rather fat well-nourished persons may be a locally conditioned relative deficiency of EFA. The epidermis presumably derives these mainly—if not entirely—from the capillaries in the dermis some distance away and, if there are large deposits of fat in the tela subcutanea, the supply may be diminished.

Two diseases in which we have had some preliminary success with therapy with EFA are Darier's disease and pityriasis rubra pilaris. In all cases of the latter disease so far examined, serum hexaenes have been zero and the dienes and tetraenes very low. This is a field that needs further study.

5. Eczema in Infants

Cow's milk, unlike human milk, is a very poor source of EFA. Dried cow's milk, if exposed to air, is an even poorer source because of the instability of the small amount of EFA in it.[1] Deficiency of EFA might therefore be expected in

[1] Analyses of fresh and dried cow's milk for their content of polyethenoid acids are reported by James & Lovelock on p. 262 of this number of the Bulletin. For a further discussion of this controversial subject, see also H. M. Sinclair, *Lancet*, 1958, 1, 639 [Letter].—ED.

children fed on cow's milk, and in 1931 Hansen tried oral administration of oils rich in unsaturated fatty acids in the treatment of skin disorders in children. Encouraging results were obtained in cases of infantile eczema. He therefore studied the lipids in the serum of five cases of infantile eczema and discovered that the iodine number of the total fatty acids was reduced. Clinical improvement on treatment with oil was accompanied by a rise in the iodine number. He reasonably suggested that a deficiency of unsaturated fatty acids may be an aetiological factor in infantile eczema. I have recently reviewed elsewhere the evidence for and against the role of EFA in infantile eczema (Sinclair, 1957b). Hansen has recently summarized his work which leaves no doubt that deficiency of EFA can occur in children (Hansen, Adam, Wiese, Boelsche & Haggard, 1958).

6. Structural Function of Essential Fatty Acids

Certain phospholipids, particularly phosphatidylethanolamine, are normally very rich in EFA; EFA also esterify glyceryl ethers such as batyl alcohol, and cardiolipin contains about five times as much linoleic acid as oleic acid. Cholesteryl esters are also well known to be very rich in EFA under normal conditions, except in the liver where they contain oleic acid and saturated fatty acids. We studied the deposition of cholesterol and phospholipid in the epidermis of the rat deficient in EFA (Basnayake & Sinclair, 1956; Sinclair, 1957a). Both cholesterol and phospholipids are increased, and the iodine numbers of the fatty acids in the phospholipid and the cholesteryl esters are decreased, in deficiency. The increase in esterified cholesterol is greater than that in free cholesterol. We pointed out in our work that in this context "cholesterol" means Liebermann-Burchard-reacting sterols and includes lathosterol and other fast-reacting sterols. Frantz, Dulit & Davidson (1957) have shown that there is about four times as much cholesterol as lathosterol in normal rat skin and that only a quarter of the cholesterol is normally unesterified. We interpreted the accumulation of phospholipids and cholesteryl esters in epidermis in EFA-deficiency as follows. These compounds are normally synthesized by the cells in and near the epidermis and contain EFA. If EFA are deficient or if there is a relative excess of more saturated fat or unnatural isomers, abnormal forms of phospholipid and cholesteryl esters will be made. As these cannot be used for structural purposes by cells, they will accumulate, and the cell may well continue to synthesize them at an increased rate, since it is deficient in utilizable phospholipid and cholesterol. Further, these abnormal esters may not be so easily transported in the form of lipoproteins and may not be readily metabolized. Therefore, when EFA are given to a deficient animal there will be disappearance of the accumulated lipid if EFA displace the wrong type of fatty acid, and the compounds can then be used for structural purposes, synthesis will be decreased, and excretion will be slightly increased.

Cell membranes are rich in phospholipids and in free cholesterol, and they are also rich in unsaturated fatty acids. Probably the EFA in phospholipids together with cholesterol and protein form the cell membrane; in deficiency of EFA there is increased fragility of erythrocytes (Macmillan & Sinclair, 1958). Mitochondria, microsomes and cell nuclei are rich in unsaturated fatty acids, the phospholipids of these cell particles being particularly rich in arachidonic acid.

Unfortunately little work has been done on the histochemistry of skin in relation to EFA, and not much electron microscopy of the skin has yet appeared. The interesting study by Selby (1955) shows that the human and rodent dermis and epidermis are separated by a dermal membrane about 350 Å thick and a "space" (probably filled with polysaccharides) about 300 Å across before the basal cell membrane is reached. This basal cell membrane is adjacent to, if not continuous with, dense granules at which terminate the tonofilaments. Other tonofilaments sweep through the cytoplasm to end in dense double disks corresponding to the nodes of Bizzozero at the intercellular bridges in human epidermis. Selby finds no evidence of continuity of cytoplasm across the intercellular bridges. The tonofilaments are rich in a fibrous protein, Rudall's epidermin, and probably certain lipids as well; the bridges are rich in phospholipid. The epidermis is in fact unusually rich in lipid, that of the mouse having about as much lipid as protein (Wicks & Suntzeff, 1942). Kooyman (1932) found that the skin of human palms and soles, which contain no sebaceous glands, contained mainly unesterified cholesterol in the stratum basale and spinosum, and a higher proportion of esterified cholesterol in the stratum corneum. The epidermal cells are very active in synthesizing cholesterol and also esterify it; this appears to accompany keratinization. During keratinization the phospholipids are largely broken down. Probably the tonofilaments are the precursors of keratin, and keratinization starts at the nodes of Bizzozero. In the formation of lipid-rich soft keratin (epidermis, internal root sheath of hair follicles and medulla of hair), unsaturated fatty acids may be involved in binding together the keratin fibrils. In the formation of lipid-poor hard keratin (nails, and cortex and cuticle of hair), which appears to be unaffected in deficiency of EFA, polymerization appears to occur through cupric ions catalysing the formation of disulphide cross-linkages.

Keratin is constantly being shed from the stratum corneum, and in the normal process the adherent keratin fibrils disintegrate into minute particles which are desquamated. If this disintegration is inhibited, the stratum corneum is thickened and hyperkeratosis results. This name is unfortunately also given to quite a different aberration of shedding: instead of minute particles of keratin, visible scales may be shed. These scales are usually formed by an accelerated rate of keratinization, and in this case parakeratosis is usually found (as in psoriasis); or scales may be formed by cracking of the outermost layer of the stratum corneum (as in ichthyosis). There is no parakeratosis in deficiency of EFA, and the stratum corneum is very compact and does not shed normally; this is probably because EFA are required for the normal binding together of keratin fibrils.

7. Permeability of the Skin in Deficiency of Essential Fatty Acids

In 1951 Miss Buxton (now Mrs Sayer) and I observed that if EFA-deficient rats were placed in glass beakers, the walls quickly grew misty through condensation of water, which suggested that the skin was losing water at an abnormal rate. Further evidence was obtained (Sinclair, 1952) by immersing rats in water for two hours: EFA-deficient animals invariably gained weight, and controls invariably lost weight. Application to the skin of toxic substances such as barium sulphide killed EFA-deficient rats but not controls. Subsequently direct proof was quantitatively obtained for the increased

passage of water or Ringer's solution through the skin of the rat (Basnayake & Sinclair, 1954, 1956) and mouse (Macmillan & Sinclair, 1958).

The permeability of the skin has been studied by various workers, and some of these investigations have been reviewed by Calvery, Draize & Laug (1946), by Cameron & Short (1948) and by Rothman (1943, 1954). Two points of interest are the site and the nature of the barrier to diffusion of water through the skin. The site is generally agreed to be the stratum granulosum (Blank, 1953; Treherne, 1956); the skin of the frog, which has no transitional layers, is freely permeable to water. The cholesterol–phospholipid framework of cells might be responsible for water's failing to permeate as easily as lipid-soluble substances: Starkenstein and his colleagues showed that breaking this framework increases the permeability of the skin to water (Starkenstein & Hendrych, 1936; Matschak, 1936), and saponins which precipitate cholesterol act similarly (Milbradt, 1933). Treherne (1956) has shown that the barrier in rabbit skin is lipoidal in nature and has the form of an aqueous phase separating two lipid layers.

Before the site of the barrier had been defined as the stratum granulosum, and before the existence of intercellular bridges with continuity of cytoplasm in the stratum spinosum had been discarded (Selby, 1955), I had supposed that the acanthosis was responsible for the increased permeability to water of the skin of the EFA-deficient animal (Sinclair, 1952). This view is no longer tenable. Certainly there are structural changes also in the stratum granulosum as Ramalingaswami and I described (1953): in the EFA-deficient rat this layer becomes composed of three to four layers of cells instead of the normal single flattened layer; its cells become packed with numerous irregular densely staining granules which are small and rounded in the deeper layers but assume large ovoid shapes in the superficial layers. At present it is not possible to explain the functional change in terms of the structural abnormality; electron microscopy of the transitional layers of the epidermis in normal and EFA-deficient animals is required.

Obviously this increased loss of water from the skin will have effects upon the animal. It will be exaggerated by dry atmospheres, and these are known to increase the severity of the dermatosis, as mentioned already. Burr & Burr (1930) first observed that EFA-deficient rats drank more water than did controls. Many authors have confirmed and extended this observation, and have shown that the loss of water in urine is decreased rather than increased, and that the water content of the carcass and of various organs is normal. When we first observed objectively the increased loss of water through the skin I suggested (Sinclair, 1952) that this might account for the raised metabolic rate of EFA-deficient animals and for their increased caloric intake. When my colleague, Dr Basnayake, measured the actual rate of passage of water through the skin, it became possible to calculate this caloric and water deficiency caused by increased permeability. Such a calculation on mice is given by Macmillan & Sinclair (1958), where it is shown that the waste of calories is greater than can be accounted for by the skin lesion alone. Since there is uncoupling of oxidative phosphorylation in deficiency of EFA (Klein & Johnson, 1954), this is the probable explanation. Panos, Finerty, Klein & Wall (1958) have demonstrated that this uncoupling is not caused by hyperthyroidism, and we have confirmed this (Macmillan & Sinclair, 1958). It is probably caused by defective mitochondrial structure. So we have yet another structural function of essential fatty acids.

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ESSENTIAL FATTY ACIDS AND HUMAN DISEASE

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1 The essential fatty acids
2 Coronary artery disease
3 Evidence contrary to the essential fatty acid theory
4 Discussion
5 Idiopathic hypercalcaemia of infancy
6 Conclusions
References

One of the great triumphs of medical science was the demonstration that some human disorders were due to lack of certain essential dietary constituents caused by the consumption of either an inadequate or an unbalanced diet. For this reason many pathological conditions of the apparently well nourished, not obviously due to an infectious process, have been blamed on lack of some unknown essential dietary factor. In recent years an insufficient intake of the "essential" fatty acids has been suggested as the cause of many diseases, in particular coronary artery disease and idiopathic hypercalcaemia of infancy.

1. The Essential Fatty Acids

The young of certain animal species (notably the rat), when fed on diets free of all fat, develop certain characteristic skin lesions, fail to thrive and often die. These symptoms can be alleviated by adding to the diet small amounts of certain poly-unsaturated acids, viz., linoleic acid (octadeca-9 : 12-dienoic acid) and arachidonic acid (eicosa-5 : 8 : 11 : 14-tetraenoic acid). A third substance, linolenic acid (octadeca-9 : 12 : 15-trienoic acid), causes remission of some symptoms but is not so effective as the other two acids. The structure of these acids is as follows:

Linoleic acid
$CH_3(CH_2)_4CH{=}CH{-}CH_2{-}CH{=}CH{-}(CH_2)_7CO_2H$

Linolenic acid
$CH_3CH_2CH{=}CH{-}CH_2{-}CH{=}CH{-}CH_2{-}CH{=}CH{-}(CH_2)_7CO_2H$

Arachidonic acid
$CH_3(CH_2)_4CH{=}CH{-}CH_2{-}CH{=}CH{-}CH_2{-}CH{=}CH{-}CH_2{-}CH{=}CH{-}(CH_2)_3CO_2H$

All three acids are found in natural fats, certain vegetable oils being particularly rich in linoleic or linolenic acids, whilst arachidonic acid is found only in animal fats. We have analysed, in collaboration with Miss J. Webb, the fatty acid composition of some typical food fats and also of some fats of animal origin; the results are shown in Tables I and II.

Whilst it is true that Hansen, Adam, Wiese, Boelsche & Haggard (1958) have shown that human babies develop skin symptoms similar to those of the rat when fed on diets deficient in fat, the lesions in the rat do not resemble those of any known human disease nor do the experimental conditions approximate to any natural dietary regime. The results do indicate, however, that the young of some animal species require a certain minimum intake of linoleic or arachidonic acids.

2. Coronary Artery Disease

This disease, or rather group of diseases, has become one of the most important causes of death among middle-aged men in western countries. The demonstration that atheromatous lesions, said to precede the coronary occlusion, are predominantly lipid in character has naturally focused attention on the possibility of a relationship between dietary fat and the disease. Epidemiological studies have produced an impressive body of circumstantial evidence relating the incidence of the disease in different social and regional groups to the total intake of dietary fat.[1] On considerably more slender evidence it is suggested that coronary artery disease and other human disease is a consequence of a deficiency of essential fatty acids in the diet (Sinclair, 1956a).

Some animals, for example, the rabbit, develop atheromatous lesions when fed diets containing large amounts of cholesterol. Some reduction in the severity of these lesions occurs if vegetable oils rich in poly-unsaturated acids are consumed simultaneously with the cholesterol. In addition there appears to be an association between the plasma cholesterol concentration in man and the incidence of coronary artery disease, particularly when comparisons are made between

[1] See Bronte-Stewart, p. 243 of this number of the Bulletin.—Ed.

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different regional and social groups and between the sexes. For a recent review see the paper by Gubner (1957).

This association is difficult to establish in a homogeneous population; nevertheless it is commonly assumed that a high plasma cholesterol concentration is directly related to the incidence of coronary artery disease. If this assumption is accepted, then the disease can be related to the intake of essential fatty acids by the following experimental evidence.

Human subjects, particularly from indigenous populations of tropical countries whose diet contains little fat and who possess normally low plasma cholesterol levels, show a rise in this component when fed large quantities of animal fats, but no change when fed similar amounts of vegetable fats. Furthermore the action of animal fats in raising the level of serum cholesterol can be neutralized by the simultaneous feeding of vegetable or fish oils (Bronte-Stewart, Antonis, Eales & Brock, 1956). Food fats of animal origin are often low in poly-unsaturated acids and high in saturated acids, whereas vegetable or fish oils are usually predominantly unsaturated (see Table I).

TABLE I. FATTY ACID COMPOSITION OF SOME FOOD FATS

Acid	Cow's milk (%)	Margarine A (%)	Margarine B (%)	Maize-oil (%)	Olive-oil (%)	Beef-dripping (%)
n-C_8	0.6	1.3	1.3	—	—	—
n-C_{10}	2.3	1.2	1.2	—	—	0.13
n-C_{12}	2.4	16.0	11.2	—	—	0.23
n-C_{14}	9.7	6.5	6.5	—	—	3.5
Mono-unsaturated C_{16}	2.3	0.8	3.4	0.2	0.9	3.9
n-C_{16}	22.5	22.2	21.9	11.7	11.3	23.2
Di-unsaturated + tri-unsaturated C_{18}	3.4	8.9	9.0	54.8	8.3	1.5
Mono-unsaturated C_{18}	25.2	33.0	30.6	31.2	75.8	28.5
Isomers of oleic	6.5	4.1	8.5	—	—	5.5
n-C_{18}	16.2	5.9	6.5	2.0	3.9	23.2
First unsaturated "C_{19}"	1.4	—	—	—	—	—

3. Evidence Contrary to the Essential Fatty Acid Theory

Apart from the work of Hansen *et al.* (1958), already mentioned, there is no unequivocal evidence demonstrating that a deficiency of linoleic and arachidonic acids in man can cause a disease. It is particularly unsound to extrapolate from animal experiments to man where nutritional deficiency effects are concerned. Recent work (James, Lovelock & Webb, 1957) has shown that human red blood cells can synthesize both acids from acetate, whilst rat red cells do not appear able to do so. In passing, it might be noted that it is almost impossible to deplete adult rats of essential fatty acids by dietary manipulation. Furthermore the experimental diets used to precipitate the deficiency state in young animals in no way resemble any normal human diet. It is only in certain primitive communities in tropical regions that diets very low in fat are common, and the available evidence indicates that there the incidence of coronary artery disease is lower than in western countries.

Essential fatty acid deficiency in experimental animals is normally associated with a low level of blood cholesterol, similar levels being found in human beings on diets low in fat.

TABLE II. FATTY ACID COMPOSITION OF FATS OF ANIMAL ORIGIN

Acid	Rat red cells (total lipids)	Human red cells (total lipids)	Human α-lipoprotein cholesteryl esters	Human β-lipoprotein phospho-lipids
n-C_{12}	0.6	0.3	—	0.5
Mono-unsaturated C_{14}	—	—	—	2.8
n-C_{14}	1.03	0.6	1.08	0.9
Mono-unsaturated C_{16}	1.3	0.83	3.6	2.6
n-C_{16}	31.3	20.4	9.4	31.3
Di-unsaturated + tri-unsaturated C_{18}	10.6	13.3	56.5	10.2
Mono-unsaturated C_{18}	14.8	22.2	22.0	17.4
Isomers of oleic	0.9	—	—	—
n-C_{18}	17.7	14.6	0	18.5
First unsaturated "C_{19}"	—	3.6	—	—
Arachidonic	17.0	16.3	5.3	6.2
Poly-unsaturated C_{20}	1.6	3.6	—	5.0

Assuming that blood cholesterol concentrations are important, this would suggest an inverse relationship between the dietary intake of essential fatty acids and coronary artery disease.

The principal basis of the alleged correlation between essential fatty acids and coronary artery disease in man is the observation of many workers, particularly Bronte-Stewart *et al.* (1956) and Ahrens, Blankenhorn & Tsaltas (1954), who have demonstrated that the feeding of certain animal fats raises blood cholesterol, whereas the feeding of vegetable oils rich in linoleic acid does not. The correlation thus rests on the following chain of argument. Since high levels of blood cholesterol are reputedly associated with a high incidence of the disease, any diet which will lower blood cholesterol (some vegetable oils) will lower the incidence, and any diet which raises blood cholesterol (some animal fats) will increase the incidence.

Evidence contrary to this chain of argument comes from the observations of Bronte-Stewart, Keys & Brock (1955) in particular. These authors compared the incidence of coronary artery disease and blood cholesterol levels in Bantu and in coloured and white inhabitants of Cape Town with the fat intake of these three groups. Their results are summarized in Table III. Also shown in the table are the approximate daily intakes of certain saturated and unsaturated acids calculated from the author's data on the assumption that the vegetable oils used had a composition midway between ground-nut and sunflower-seed oils and that the composition of the animal fat lay between that of butter and beef-fat.

Table III illustrates the fact that, while high blood cholesterol levels and coronary artery disease are associated with a high intake of fat, the linoleic acid intake is actually higher in the white group than in the Bantu group. Furthermore there is no suggestion that the ratio of linoleic acid to saturated acids or indeed the ratios of any of the fatty acids are markedly different in the three groups.

Similar comparisons are made in Table IV between the fatty acid intake of Bantu fed with different fats (Bronte-Stewart *et al.* 1956), in which cholesterol changes were observed. The table demonstrates that raised cholesterol

TABLE III. COMPARISON OF INCIDENCE OF CORONARY ARTERY DISEASE IN BANTU AND IN COLOURED AND WHITE INHABITANTS OF CAPE TOWN WITH THEIR BLOOD CHOLESTEROL LEVELS AND THEIR INTAKE OF FAT (based on results of Bronte-Stewart, Keys & Brock, 1955)

	Subjects		
	Bantu	Coloured	White
Calories/day derived from fat	450	720	855
Vegetable fat (g./day)	10	20	15
Animal fat (g./day)	40	60	80
Fatty acids (g./day):			
Palmitic	10.2	16	20
Stearic	8.3	12.6	16.5
Oleic	18.5	31	34
Linoleic	3.0	5.6	4.9
Plasma cholesterol (mg./100 ml.)	166	204	234
Incidence of ischaemic heart disease	Very low	Low	High

levels are associated with diets low in linoleic acid. However, the table also shows that high levels of blood cholesterol could also be attributed either to the content of the shorter-chain saturated fatty acid (C_{10}–C_{14}) or to the stearic acid content. Thus the effects of these diets in changing cholesterol levels could as well be attributed to an excessive consumption of saturated acids as to a deficiency of linoleic acids.

The concept that a prolonged deficiency of essential fatty acids leads to development of coronary artery disease would lead one to expect a demonstrable deficiency of these acids in sufferers from this disease. Accordingly a study was made of the fatty acid composition of the blood lipids from twelve patients suffering from the disease and twelve controls matched for age and sex (James, Lovelock, Webb & Trotter, 1957). Analyses were also made by gas–liquid chromatography of the fatty acids from the lipids of the red cells, the plasma phospholipids and the plasma neutral fat (cholesteryl esters and triglycerides) as well as the total cholesterol levels in the plasma. The results are summarized in Tables V, VI and VII. It can be seen that none of the plasma lipids showed any deficiency of linoleic+linolenic acids or arachidonic acid in the diseased group as compared with the normal group. These results cannot be reconciled with any theory of essential fatty acid deficiency in coronary artery disease.

TABLE IV. COMPARISON OF BLOOD CHOLESTEROL LEVELS IN BANTU GROUPS FED WITH DIFFERENT FATS OR DIETS (based on results of Bronte-Stewart, Antonis, Eales & Brock, 1956)

Dietary fat	g./day	Content of fatty acids (%)					Rise and fall of blood cholesterol concentration compared with normal Bantu level (%)
		C_{10}–C_{14} acids	Palmitic	Stearic	Oleic	Linoleic	
Vegetable oils:							
Olive-oil	100	0	11	4	76	8	0
Olive-oil	200	0	11	4	76	8	0
Ground-nut oil	100	0.3	10	2.7	62.5	24	0
Hydrogenated nut-oil	100	3	14	8	71	4	+30
Animal fats:							
Butter	100	14	22	15	25	3	+40
Dripping	100	4	23	23	29	1.5	+25
Herring-oil	100	13	21	0.4	15	6	0
Mixed fats:							
Dripping (100 g.) + sunflower-seed oil (100 g.)	200	2	15	13	43	17	0
Cape Town diets:							
White	95	7	20	17	34	5	+41
Coloured	80	6	16	13	31	6	+23
Bantu	50	4	10	8	19	3	0

TABLE V. FATTY ACID ABUNDANCE OF THE LIPIDS OF RED BLOOD CELLS FROM PATIENTS WITH ISCHAEMIC HEART DISEASE AND FROM CONTROLS (James, Lovelock, Webb & Trotter, 1957, Table III)

Acid	Mean of nine patients (%)	Mean of six controls (%)	P*
Myristic	0.85	0.83	0.9
Palmitic	23.0	22.4	0.9
Stearic	16.6	14.7	0.75
Palmitoleic	1.2	1.1	0.9
Oleic	21.3	19.8	0.9
Linoleic	11.3	12.1	0.9
Arachidonic	14.1	13.3	0.8

* *P* indicates the probability that the observed difference between the two means is due to chance.

4. Discussion

The assumed relationship between low essential fatty acid intake and coronary artery disease rests upon two proved claims: firstly an inverse relationship between linoleic acid intake and blood cholesterol levels in man, and secondly an association between low essential fatty acid intake and atheroma in animals.

Gubner in a recent review (1957) has summarized the evidence relating atheroma in man to coronary artery disease and makes the comment that there appears to be only a slight correlation between the presence of atheroma and that of coronary artery disease. He emphasizes that the important event leading to an infarct is the development of thrombi in the coronary vessels and not merely atheroma. Lipids play

TABLE VI. ABUNDANCE OF FATTY ACIDS IN PLASMA PHOSPHOLIPIDS OF PATIENTS WITH ISCHAEMIC HEART DISEASE AND OF CONTROLS (James, Lovelock, Webb & Trotter, 1957, Table IV)

Acid	Mean of 12 patients (%)	Mean of 12 controls (%)	P*
Myristic	1.1	1.1	0.9
Palmitic	25.5	26.7	0.9
Stearic	13.4	13.3	0.9
Palmitoleic	1.94	2.03	0.9
Oleic	20.7	20.8	0.9
Linoleic	19.6	18.8	0.9
Arachidonic	9.6	9.7	0.9

* *P* indicates the probability that the observed difference between the two means is due to chance.

TABLE VII. FATTY ACIDS, CHOLESTEROL, AND GLYCERIDE-GLYCEROL OF THE ACETONE-SOLUBLE FRACTION OF PLASMA LIPIDS IN PATIENTS WITH ISCHAEMIC HEART DISEASE AND IN CONTROLS (James, Lovelock, Webb & Trotter, 1957, Table V)

Lipid component	Mean composition in 12 patients	12 controls	P*
Mean abundance of fatty acids:			
Myristic	3.5%	4.7%	0.1
Palmitic	23.9%	24.1%	0.9
Stearic	6.9%	7.7%	0.3
Palmitoleic	5.3%	4.6%	0.1
Oleic	38.1%	34.7%	0.06
Linoleic	12.1%	12.4%	0.9
Arachidonic	2.7%	2.6%	0.9
Total mono-unsaturated acids C_{14}, C_{16}, and C_{18}	45.9%	41.6%	0.05
Other lipid components (mg./100 ml. of plasma):			
Cholesterol	197.6	189.7	0.8
Glyceride-glycerol	4.4	4.0	0.6
Ratio of oleic acid to stearic acid	5.8	4.6	0.02

* *P* indicates the probability that the observed difference between the two means is due to chance.

an important part in blood coagulation and, so far as this effect is concerned, a high plasma cholesterol level may even be beneficial, since cholesterol can inhibit coagulation. Furthermore O'Brien (1956) has demonstrated that the important lipid components in coagulation are the phospholipids, in particular phosphatidyl ethanolamine.

The evidence relating fat intake and the total caloric intake to coronary artery disease, whilst impressive, cannot be the whole story, since young women (whose diets cannot be very dissimilar to those of men) appear to be immune from the disease.

Lipid metabolism in the human subject is a complex biochemical problem that has so far received too little attention. Most earlier workers have for obvious reasons studied those lipids whose quantitative determination is relatively easy, for example cholesterol and phospholipids. It is probable that the lipid changes leading to coronary occlusions will be reflected in disturbance in nature or level of many of the normal blood lipids. Some of these changes may be only secondary in nature, as Gubner has suggested for the raised plasma cholesterol level, and not a primary cause of the disease. However, at this stage of development in the study of coronary artery disease it can be stated unequivocally that there is no adequate evidence to show that a deficiency of essential fatty acids is a factor in its causation.

TABLE VIII. LEVELS OF LINOLEIC + LINOLENIC AND OF ARACHIDONIC ACIDS IN WHOLE BLOOD OF THREE INFANTS WITH HYPERCALCAEMIA, COMPARED WITH THOSE OF THREE NORMAL CHILDREN AS CONTROLS

		Serum calcium (mg./100 ml.)	Linoleic and linolenic acids as percentage of total blood fatty acids	Arachidonic acid as percentage of total blood fatty acids
Control 1	No hypercalcaemia	10.7	9.4	5.4
Control 2		10.6	10.3	4.8
Control 3		11.7	10.6	4.3
Case 1	Suffering from hypercalcaemia	15.8	8.6	3.4
Case 2		13.8	8.6	4.6
Case 3		13.2	9.9	4.6

5. Idiopathic Hypercalcaemia of Infancy

Idiopathic hypercalcaemia of infancy has attracted attention in the last few years. It is characterized by a failure to thrive, by anorexia and constipation, and in some cases by an elflike face; at some stages the serum calcium and serum cholesterol levels are raised. In the United Kingdom the disease is often associated with a diet of processed cow's milk and, in particular, National Dried Milk. Sinclair (1956b) suggested that infantile hypercalcaemia was due to ingestion of fat which lacks linoleic acid, the processing of the cow's milk (by passage over hot rollers) being blamed for the loss of the small amount originally present in the milk.

TABLE IX. MAJOR COMPONENTS OF MILK-FATS FROM A VARIETY OF SOURCES, EXPRESSED AS PERCENTAGE OF FATTY ACIDS IN THE RANGE C_6–C_{20}

Acid	Human milk sample 1, ten days after commencement of lactation	Human milk sample 2, three months after commencement of lactation	Fresh cow's milk	Roller-dried milk	National Dried Milk	Evaporated milk
Myristic	5.7	9.0	—	10.7	10.6	9.4
Branched C_{15}	0.5	0.2	0.8	1.3	1.6	1.8
n-C_{15}	0.8	0.4	1.0	1.3	1.2	1.4
Palmitoleic	3.9	2.6	2.2	2.2	2.0	2.4
Palmitic	26.5	20.0	26.4	29.7	16.6	21.8
Branched unsaturated C_{17}	1.5	0.8	1.5	1.5	2.0	1.5
n-C_{17}	0.7	Trace	0.7	1.1	0.6	0.8
Linoleic + linolenic	5.1	4.4	3.2	3.5	4.1	4.4
Oleic	38.6	46.0	23.3	26.1	30.4	26.0
Isomers of oleic acid	Not measured	Not measured	3.6	3.1	10.0	5.1
Stearic	12.1	7.7	10.3	12.0	10.5	13.8
Poly-unsaturated C_{20} (not arachidonic)	1.8	Not measured	—	—	1.0	2.0

If this were true, it should be possible to demonstrate a deficiency of linoleic and arachidonic acids in the blood of children suffering from the disease. Investigation of this point (James, Webb, Stapleton & Macdonald, 1958), however, revealed no significant difference between the levels of these acids in whole blood of three infants with the disease and in the blood of three normal children (see Table VIII). Furthermore, a comparison of the fatty acid composition of the fat of fresh cow's milk with that of roller-dried milk, National Dried Milk and of evaporated milk showed that no significant change in linoleic acid + linolenic acid content occurred during the drying processes. Comparative analyses of two samples of human milk demonstrated that the linoleic and linolenic acid content was not much greater than that of cow's milk (see Table IX). These results do not support the concept that a deficiency of essential fatty acids is a factor in the causation of infantile hypercalcaemia.

6. Conclusions

There is little satisfactory evidence connecting the dietary intake of essential fatty acids with either coronary artery disease or infantile hypercalcaemia. It has yet to be proved that any other human disease is due to a deficiency of essential fatty acids.

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LIPIDS AND THE STRUCTURE OF MYELIN

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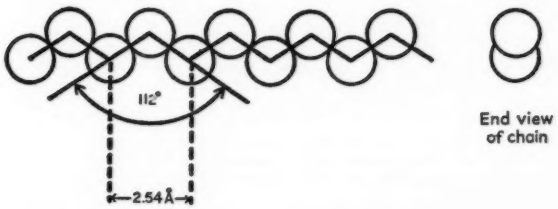
1 Structural characteristics of lipids
2 General features of myelin structure
3 Origin of the myelin sheath
4 Chemical composition and molecular structure of myelin
References

Indications of the prominence of lipid components in nerve myelin were found in the earliest histological and chemical studies of nerve tissue. Details of the different types of lipid molecules incorporated and their structural interrelationships have evolved slowly, following the application of refined chemical analytical techniques and methods of structural analysis such as polarized light microscopy, *x*-ray diffraction, and electron microscopy.

1. Structural Characteristics of Lipids

Lipid molecules in general tend to form layer structures, the long hydrocarbon chains associating along their lengths by means of van der Waals' forces, and the end-groups are all in the same plane. The simplest example of this layered structure is probably that formed by polar lipids on a water surface. In compressed lipid monolayers the ionic end-groups are directed into the water surface, and the axes of the hydrocarbon chains are oriented at right angles to the surface. From studies of these surface monolayers, it was suggested that the hydrocarbon chain adopted a zigzag configuration (Langmuir, 1917). This was later confirmed for crystalline lipid materials by *x*-ray diffraction studies which showed the angle of the zigzag to be 112°, thus giving a 2.54-Å repeat (comprising two carbon atoms) along the chain (Müller, 1927, 1928; Bunn, 1939). The characteristics of the hydrocarbon chain are thus as indicated in fig. 1. *x*-Ray diffraction studies also showed that polar lipids tend always to form double layer repeating units, the polar ends of the molecules coming into opposition. The interrelationships of the end-groups in the layered structure were also revealed in single crystal analyses of paraffin hydrocarbons and of fatty acids, but single crystal studies of more complex lipids are not yet available. However, studies of crystalline powders of other lipid materials have indicated that the zigzag configuration is normal for the hydrocarbon chain and that, in general, asymmetric lipid molecules form the double layer repeating unit (Malkin, 1952). It has also been demonstrated that many lipids can exist in different polymorphic forms, the main variable being the angle of tilt of the long axis of the molecule with respect to the plane of the layers (Malkin, 1952). Many polar lipids were found to incorporate water at the polar interfaces between layers, in some cases to the extent of introducing a water layer of a thickness greater than that of the double lipid layer itself (Palmer & Schmitt, 1941). This tendency of the polar lipid materials to incorporate water is responsible for the formation of myelin forms, the highly birefringent, tube-like structures which grow out from a lipid phase in contact with water. In the presence of water, different types of lipid molecules will associate to form mixed lipid layers.

FIG. 1. DIAGRAM SHOWING THE IMPORTANT PARAMETERS OF THE ZIGZAG ARRANGEMENT OF CARBON ATOMS IN THE HYDROCARBON CHAIN



2. General Features of Myelin Structure

This basic information concerning the structural characteristics of lipid molecules has greatly facilitated considerations of the complex myelin structure. When quantitative data on the birefringence of the myelin sheath were obtained, the radial component could be readily accounted for in terms of lipid layers arranged so as to orient the long axes of the lipid molecules in the radial direction. However, it was found that, in order to explain the whole of the birefringence data, a further factor providing a weakly birefringent component directed perpendicular to the radius of the nerve fibre was necessary. This was suggested to be in the form of a thin protein layer intercalated between the lipid layers. W. J. Schmidt (1936) proposed a lipoprotein organization of the type shown in fig. 2.

FIG. 2. SCHEMATIC DRAWING OF MYELIN SHEATH STRUCTURE, AS DEDUCED FROM POLARIZED LIGHT DATA BY W. J. SCHMIDT (1936)

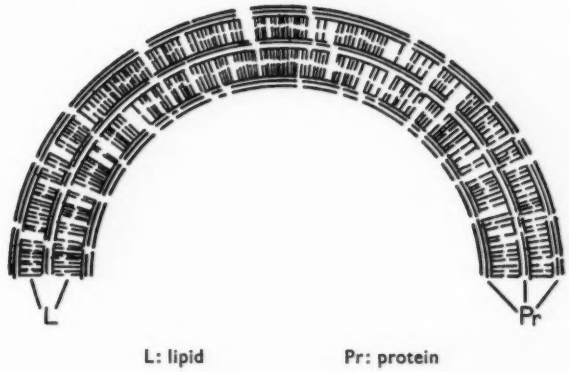
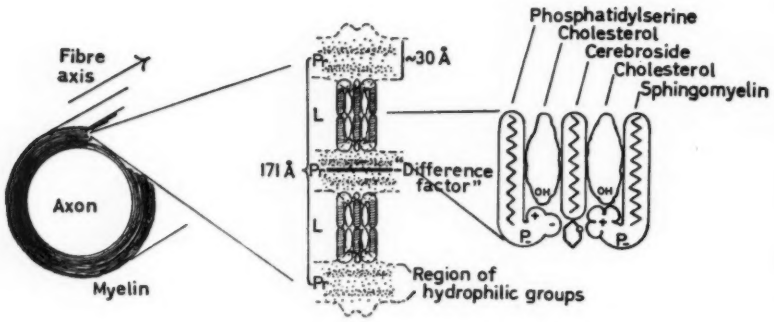


FIG. 3. DIAGRAM SUMMARIZING STRUCTURAL AND CHEMICAL FINDINGS RELATING TO THE MYELIN SHEATH OF FROG SCIATIC NERVE

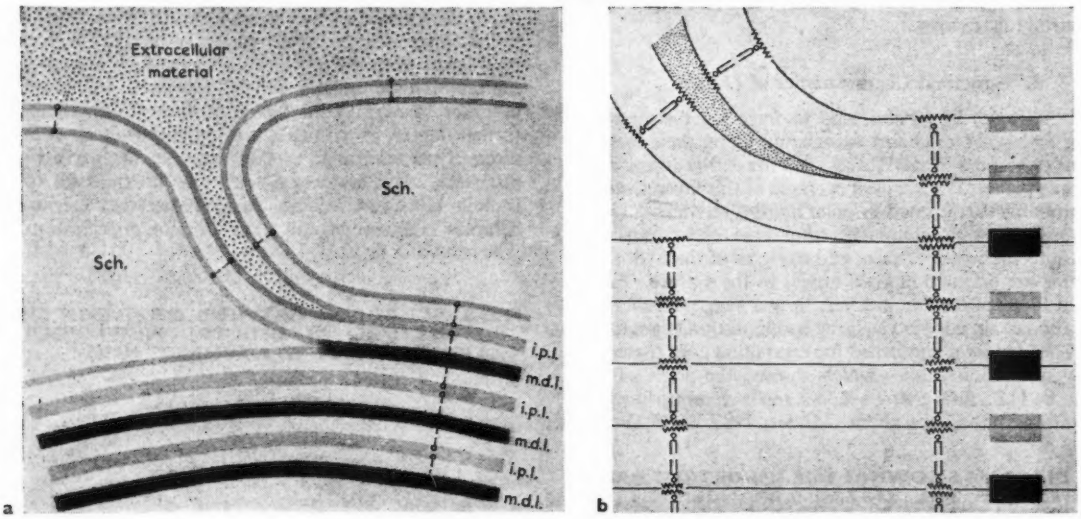


L: lipid
Pr: protein
P: phosphorus

The wide-angle *x*-ray diffraction pattern of nerve, first recorded by Boehm (1933), could be interpreted in terms of radially oriented lipid molecules. When the low-angle reflections were eventually recorded by Schmitt, Bear & Palmer (1941) and Schmitt (1950), the radial repeat was seen to be about 170 Å for amphibian peripheral nerve, and 180–185 Å for mammalian peripheral nerve. It was suggested that such a repeat must include two bimolecular lipid leaflets, and several ways of arranging lipid bimolecular leaflets and thin protein layers to give such a unit were devised. The study of the low-angle diffraction patterns of myelin was extended by Elkes & Finean (1949, 1953a, 1953b) who produced modifications of the structure by controlled dehydration, by temperature changes, and by treatment of the nerve fibres with lipid solvents. The observations all pointed to a lipoprotein association in which part of the lipid was fairly labile and readily separated from the protein, whilst part of it was relatively firmly bound. Finean (1953a) eventually gave reasons for preferring an alternation of double layers of lipid, 50–55 Å thick, and thin (30 Å) layers of protein, alternate layers probably differing in terms of some specific groups identified as the "difference factor" (Finean, 1956) (see fig. 3).

Meanwhile, Fernandez-Moran (1950, 1954) had obtained electron micrographs showing a system of radially repeating layers in the myelin sheath. The nerve preparations, from which these electron micrographs were made, were fixed in osmium tetroxide and were sectioned while frozen and then dried. In these preparations dense lines were repeating radially at intervals of 60–70 Å, but in the material embedded in

FIG. 4. DIAGRAM OF JUNCTION OF SCHWANN CELL AND MYELIN LAMELLAE

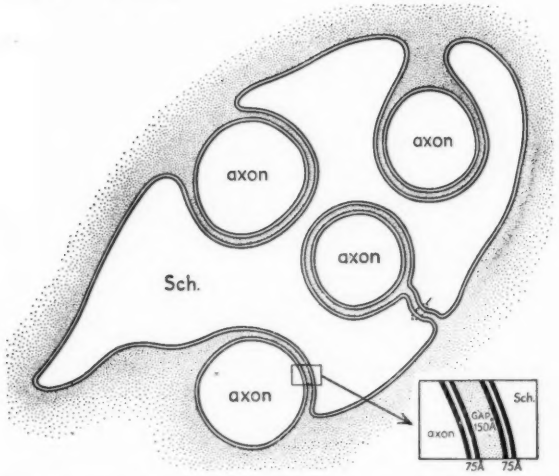


In (a) the Schwann-cell (Sch.) unit membrane forming an outer mesaxon in adult myelin is shown at its junction with compact myelin. The outermost intraperiod line (i.p.l.) is seen to be formed by the contact of the outer surfaces of the unit membranes with the extrusion of the gap substance. The heavy (major) dense lines (m.d.l.) are formed by contact of the (inner) cytoplasmic surfaces of the unit membranes. (The same relative density distributions are seen after both potassium permanganate- and osmium tetroxide-fixation but the outer surfaces of the unit membranes outside compact myelin are seen clearly only after fixation with potassium permanganate.) The bimolecular lipid layers presumed to be in the unit membranes before fixation are indicated (○/○). In (b) the crucial region of junction of the mesaxon with compact myelin is shown at greater magnification. The bimolecular leaflets of lipid and layers of non-lipid material of each myelin lamella and each unit membrane are indicated (see also fig. 3). The relative densities of the various layers in compact myelin are shown to the right, as they appear after fixation with potassium permanganate. (Fixation with osmium tetroxide gives similar densities but the intraperiod lines are less regular.)

methacrylate, subsequently examined by Sjöstrand (1953), there was a marked alternation in the densities of the lines, the fundamental repeating period being about 120 Å. Sjöstrand's micrographs showed major dense lines ~ 25 Å thick separated by light zones of ~ 95 Å, the latter being bisected by medium dense but less regular lines, now commonly called intermediate or intraperiod lines. Finean (1954, 1958), and Fernandez-Moran & Finean (1957), made *x*-ray diffraction studies of the changes in myelin structure produced by the preparative techniques used in electron microscopy. Such studies clearly indicate that the 120–130 Å periodic structure seen in the electron microscope is derived from the 170–180 Å unit detected in fresh myelin (Fernandez-Moran, 1957, 1958; Finean, 1957a; Robertson, 1957c).

The relationship between the density variations observed in the electron micrographs and the chemical composition of the myelin sheath is still uncertain. From *x*-ray diffraction data it is suggested that fixation with osmium tetroxide markedly alters the density distribution in the system, probably by the addition of osmium (Finean, 1954). Osmium tetroxide is, however, capable of reacting with a variety of chemical groupings on both lipid and protein molecules (Bahr, 1954, 1955), so that the sites of deposition of osmium in this system are difficult to predict. Nevertheless, there is indirect evidence (Fernandez-Moran & Finean, 1957; Finean, 1957a, 1957c) to suggest that the dense lines seen in electron micrographs of myelin preparations, fixed with osmium tetroxide, cover the lipid polar groups and the protein layers (figs. 4a, 4b). In myelin preparations, fixed with potassium permanganate, the intraperiod line is much more consistent, and by prolonging the fixation its intensity can be increased until it is comparable with that of the main dense line. These dense lines in the preparations fixed with osmium tetroxide or with potassium permanganate cover identical regions (Robertson, 1957d) but it is difficult to compare their densities. Factors such as image focus (Robertson, 1958a), photographic procedures, and specimen characteristics are not yet adequately evaluated for such comparisons.

FIG. 5. DIAGRAM OF VERTEBRATE UNMYELINATED NERVE FIBRE FIXED WITH POTASSIUM PERMANGANATE



One Schwann cell (Sch.) is shown with four axons, illustrating the commonly observed types of association. The entire Schwann cell is surrounded by an amorphous-appearing material which often extends out from the surface continuously for several hundred Ångström units and into the gaps of the double membranes (see inset).

3. Origin of the Myelin Sheath

One of the most important contributions made by electron microscopy to the nerve myelin story was the establishment of an exact relationship between each of the layers demonstrated in myelin and the Schwann-cell membranes. The first step in this development was the demonstration by Gasser (1955) that unmyelinated nerve fibres of vertebrates consisted of axons simply embedded in a Schwann cell as shown in fig. 5. The enveloping lips of the Schwann cell extending around the axon are in apposition, producing a double membrane ~ 300 Å wide connecting the axon with the outside. This was referred to by Gasser by the term "mesaxon". Robertson (1957a, 1957d) has subsequently shown that the ~ 300-Å double membrane consists of two units, each ~ 75 Å wide, separated by a gap ~ 150 Å wide (fig. 5; Plate I, figs. A, C). Each of the units consists of two dense layers ~ 20 Å wide separated by a light zone ~ 35 Å wide. These unit membranes of the mesaxon are simply the surface or plasma membrane of the Schwann cell (Plate I, fig. A). The ~ 150-Å gap between the unit membranes of the mesaxon is continuous with the "basement membrane" and other extracellular material. When nerve fibres are placed in hypertonic solutions before fixation, the gap substance collapses (Plate I, fig. E (upper inset)—compare with Plate I, fig. B), bringing the two unit membranes into close contact. The gap substance is probably a highly hydrated gel, and this collapsing a process of synaeresis.

One of the most important pieces of evidence in relating myelin to cell membranes was provided by Geren (1954, 1956; Geren & Schmitt, 1954; Schmitt, 1957, 1958). Geren found intermediate forms in embryonic chick nerves in which the mesaxon was wrapped spirally about the axon as in fig. 6b and Plate I, fig. C. She postulated that adult myelin was simply a greatly elongated, spirally wound, zipped-up mesaxon. Robertson's subsequent demonstration (Robertson, 1955) of the inner and outer mesaxons in adult myelin (fig. 6 and Plate I, fig. D) gave strong support for Geren's theory. Further studies (Robertson, 1957d) have shown that the intraperiod lines result from the contact of the unit membranes of the mesaxon, perhaps by synaeresis and subsequent partial removal of the gap substance, and that the major dense lines are formed by contact of the unit membranes of the spirally wound mesaxon along their cytoplasmic surfaces. It is now quite certain that the "difference factor" required by the *x*-ray diffraction data from peripheral nerve arises from the asymmetry of the Schwann-cell membrane, demonstrated by electron microscopy. This difference factor is small in the case of normal peripheral nerve myelin and insignificant in the case of the myelin of the optic nerve, but in both systems it is greatly emphasized by fixation with osmium tetroxide.

In terms of the structure shown by the electron microscope, it may be that the heavy band and the intermediate band have only slightly different densities in the normal structure, but that one is perhaps more reactive to osmium. These osmiophilic groups are to be considered as at least part of the difference factor though not necessarily all of it.

PLATE I, FIGS. A—H

The electron micrograph shown on the plate as fig. D was made of material fixed with osmium tetroxide; all others were made of material fixed with potassium permanganate.

FIG. A. The image of a short segment of a Schwann-cell surface ~ 75-Å unit membrane is shown in three states of focus: (a) is over-focused, (b) is very close to focus, and (c) is under-focused. The out-of-focus effects in (a) and (c) are produced by the summation of Fresnel fringe intensities
(magnification × 825,000)

FIG. B. A typical double membrane of the kind seen in a wide variety of cell types including nerve fibres (see inset on fig. 5). In this instance the two ~ 75-Å unit membranes bounding two smooth muscle cells in mouse intestine are shown separated by a gap ~ 100 Å wide. Each unit membrane is like the Schwann-cell unit membrane in fig. A
(magnification × 625,000)

FIG. C. A portion of a Schwann cell (Sch.) containing an axon in a 3-day-old mouse sciatic nerve. The mesaxon (m) is wrapped spirally about the axon. The outer ~ 75-Å unit membrane of the Schwann cell is shown below, bordering extracellular substance (outside). It is enfolded to make the mesaxon (m) and here the two unit membranes are also in contact in some places
(magnification × 97,000)

FIG. D. Micrograph of lizard myelinated nerve fibre fixed in osmium tetroxide. The outer mesaxon (1), inner mesaxon (2) and axon-Schwann membrane (3) are visible. The ~ 75-Å unit membrane details do not appear in this micrograph
(magnification × 75,000)

FIG. E. Segment of frog nerve myelin after soaking in water before fixation. The outer Schwann-cell unit membrane (u) appears to the right. The outermost unit membrane of myelin is separated from the previously compact structure and lies just to the left of the outer unit. The succeeding layers into which the myelin is separated measure ~ 150 Å in width and consist of two unit membranes united along their inner (cytoplasmic) surfaces (arrow in lower inset). Inset to the upper right shows a segment of a mesaxon of an unmyelinated fibre after treatment with hypertonic Ringer solution before fixation. Here the gap between the unit membranes is collapsed by the hypertonic solution and the unit membranes are united along their outside surfaces. Normally this structure would look like the double membrane in fig. B. This type of condensed double membrane is ~ 150 Å wide, like the ~ 150-Å condensed membranes in myelin, but differs fundamentally because here the outside surfaces of the unit membranes are in contact
(magnification × 145,000; insets × 370,000)

FIG. F. High magnification of preterminal myelin region near a node of Ranvier
(magnification × 81,000)

FIG. G. Longitudinal section at node of Ranvier (frog) (magnification × 9,700)

FIG. H. Transection of node of Ranvier approximately at point indicated by vertical lines in fig. G
(magnification × 45,000)

The myelin lamellae peel off from the inside outwards as the myelin spiral unwraps over the last few microns of the internode (fig. F). The two Schwann cells extend numerous processes ~ 500 Å in diameter which interdigitate over the axon surface at the node, but do not appear to come closer to one another than 100 Å (figs. G, H). The axoplasm near the node sometimes contains multivesicular bodies such as that indicated by the arrow in fig. G. Mitochondria (m in fig. H) are also frequently observed in this region. Note apparent relation of the mitochondrion to the axon membrane

LIPIDS AND THE STRUCTURE OF MYELIN

(FIGS. A—H)

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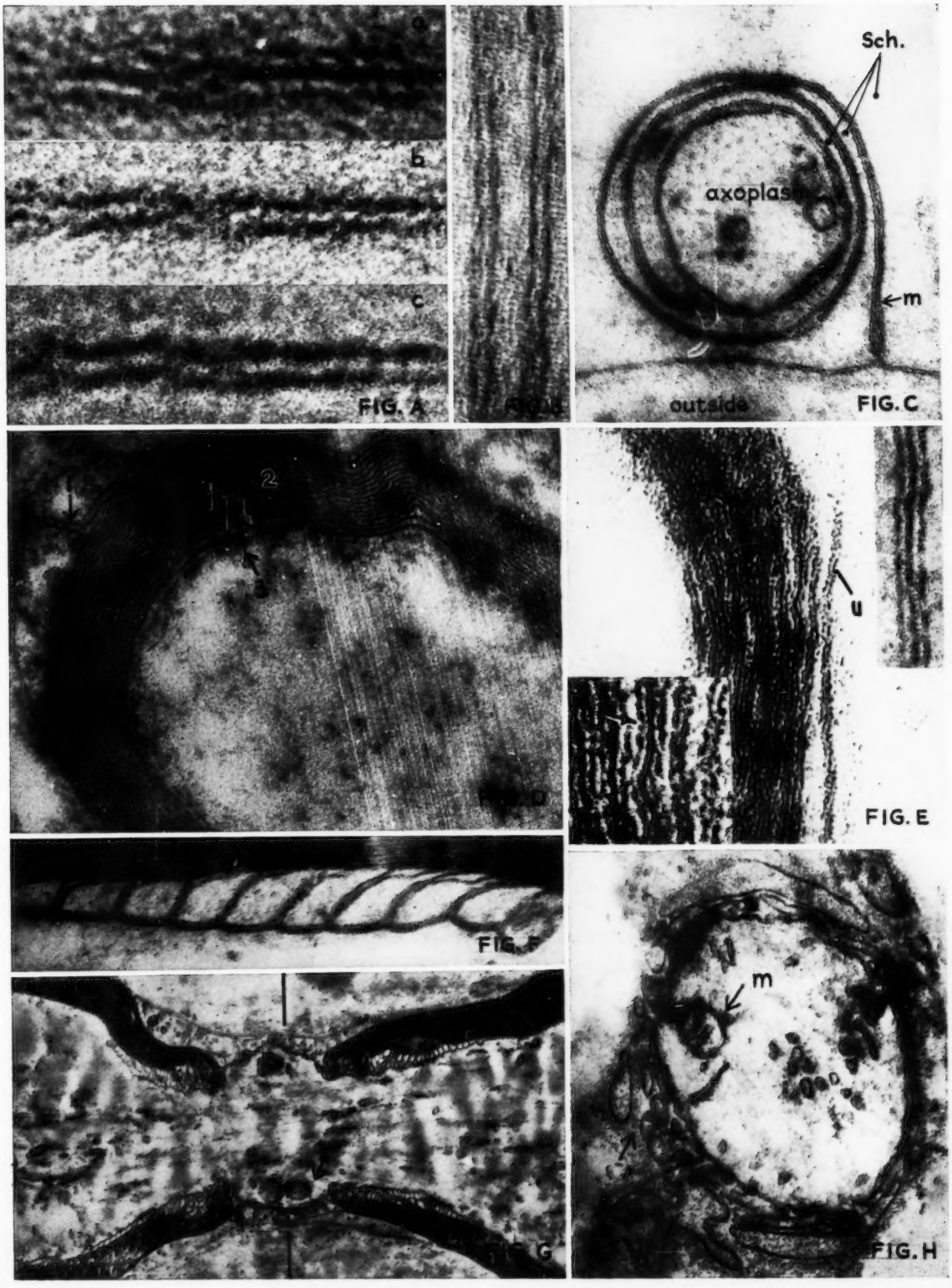
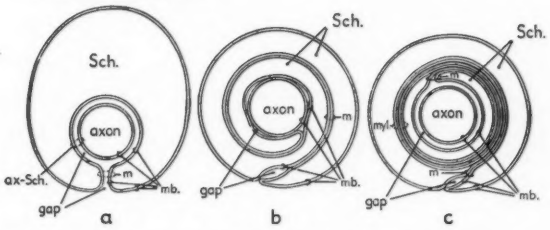
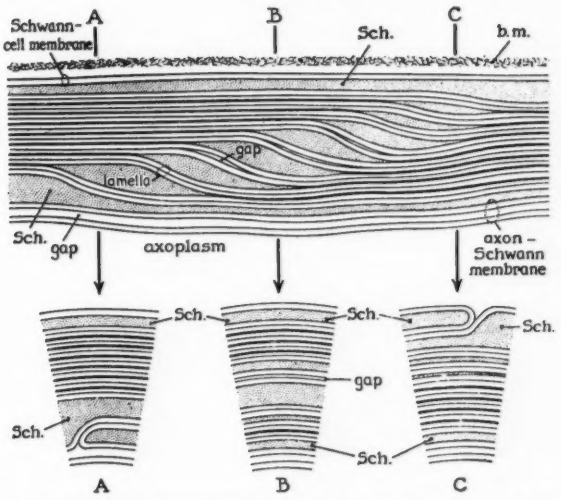


FIG. 6. DIAGRAMS INDICATING STAGES OF DEVELOPMENT OF MYELINATED FIBRES IN YOUNG MOUSE SCIATIC NERVES



(a) Shows one of the earliest stages yet recognized. An axon is enveloped by a relatively large Schwann cell (Sch.). A mesaxon (m) is present and the ~75-Å unit membranes (mb.) of both the axon–Schwann membrane (ax–Sch.) and the mesaxon are separated by 100–150-Å gaps. Sometimes the gap is partly closed as in (b). In (b) the mesaxon is elongated and surrounds the axon in a spiral. Very often the gap between the unit membrane is partially or completely closed at this stage, and the unit membranes are in close apposition along their outside surfaces, making the future intraperiod lines (see Plate I, fig. C). In (c) the Schwann cytoplasm between the mesaxon loops is excluded and the cytoplasmic surfaces of the unit membranes of the mesaxon are in contact, making the major dense line of compact myelin (myl.). The inner and outer mesaxons (m) are now visible as in Plate I, fig. D.

FIG. 7. DIAGRAM OF A TYPICAL SCHMIDT-LANTERMANN CLEFT



The ~75-Å units composing the Schwann-cell membrane and the axon–Schwann membrane are shown. The myelin lamellae are simply sheared apart in these infundibuliform structures and separate along the cytoplasmic surfaces of their component ~75-Å unit membrane, forming the major dense lines. The spaces between the lamellae are thus part of a long helical pathway connecting the outer layer of Schwann cytoplasm (Sch.) with the thin inner layer next to the axon. Sometimes the unit membranes of the lamellae are separated slightly (gap) along their outside surfaces which are normally united along the intraperiod lines of myelin. The amorphous material outside the Schwann cell is here shown as a condensed layer (b.m.).

Finean & Millington (1957) have shown that if myelin is soaked in hypotonic solutions the fundamental period increases from 170–180 Å to ~ 270 Å. Robertson (1958a, 1958c) has studied this phenomenon by electron microscopy and shown that the increase in the period is due to a separation of the unit membranes of the lamellae along the intraperiod lines (Plate I, fig. E). They do not separate along the major dense lines where they are in contact along their cytoplasmic surfaces. This behaviour alone, apart from the different ways in which the inner and outer layers react with fixatives, probably indicates that they are chemically different.

A survey of the cell membranes of a wide variety of cell types in several different vertebrates and in animals of other phyla has shown the wide-spread occurrence of this type of 75-Å unit membrane-structure, and it would appear that all such membranes probably have the same general lipoprotein architecture (Plate I, fig. B) (Robertson, 1958d). Specificities no doubt exist in the non-lipid layers or even in the lipid layers, but these are not revealed ordinarily by electron microscopy. In addition, the same ~ 75-Å unit membrane has been found in all membranous cell organelles, notably endoplasmic reticulum, mitochondria, the Golgi complex and nuclear membranes (Robertson, 1958d).

The relationships of the unit cell membranes in Schmidt-Lantermann (S-L) clefts are interesting (Robertson, 1958b). S-L clefts are funnel-shaped discontinuities in myelin in which the unit membranes are separated along the major dense lines by ~ 500–1,000 Å (fig. 7). Because of the spiral arrangement of the myelin lamellae, the large space between the separated layers constitutes a long spiral pathway of Schwann cytoplasm from the outside nucleated layer to the thin layer of cytoplasm next to the axon.

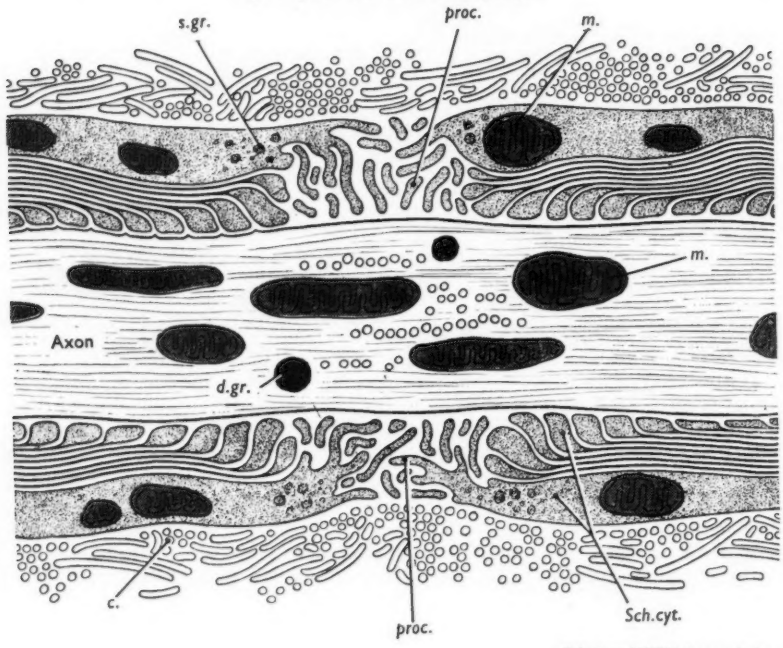
At nodes of Ranvier (Robertson, 1956, 1957b; Uzman, 1957) the arrangement of the myelin lamellae is as shown in fig. 8 and Plate I, figs. F, G. Here the spirally wrapped lamellae peel off from the inside next to the axon as the spiral unwinds, and closely spaced inner mesaxons are seen in longitudinal sections (Plate I, fig. F). The axon–Schwann membrane gaps in this region are closed. The outer mesaxon retains its orientation parallel to the fibre axis and is seen only in cross-sections. The nodal axon (~ 0.5–2.5 μ long) is unmyelinated. The axon–Schwann membrane gaps here are open (i.e., measure ~ 150 Å) (fig. 8 and Plate I, figs. G, H). The two apposed Schwann cells extend numerous finger-like cytoplasmic processes ~ 500 Å in diameter, which interdigitate over the nodal axon (fig. 8 and Plate I, figs. G, H). A definite gap of ~ 150 Å has always been seen between these processes. This gap may provide a pathway for flow of ionic current between the axon and the outside at the node.

The general organization of the myelin layers is thus demonstrated in great detail in the electron microscope, but much more information is required on what this detail means in terms of lipid and protein, and how it was related to the water component in the original tissue.

4. Chemical Composition and Molecular Structure of Myelin

Many lipid molecules have been isolated and characterized from the myelin structure. They are generally grouped as cholesterol, phospholipids, and cerebrosides, and the approximate molecular ratio between these components in myelin is 2 : 2 : 1 (Johnson, McNabb & Rossiter, 1948; Brante, 1949). In both *x*-ray diffraction and electron-microscope data there are indications of a fairly precise organization within the plane of the layers, but as yet they present insufficient information to allow structural speculations more detailed than those

FIG. 8. DIAGRAM OF A LONGITUDINAL SECTION THROUGH A NODE OF RANVIER
(compare with Plate I, fig. G)



Robertson (1957b) by permission of Journal of Physiology

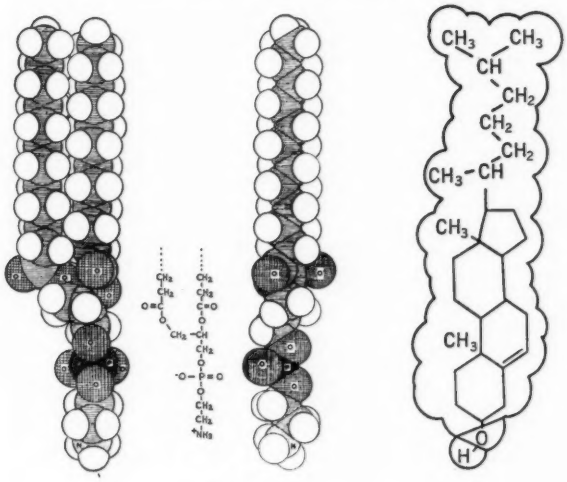
The ~75-Å membrane details are not included. The axon in the nodal region contains longitudinally disposed axon filaments, typical elongated mitochondria (m.), vesicular bodies ~500 Å in diameter (clear circles) and, in osmium tetroxide-fixed material, very dense round bodies showing no internal structure (d.gr.). The two Schwann cells (Sch. cyt.) extend numerous finger-like processes (proc.), ~500 Å in diameter, out over the non-myelinated nodal region which is ~0.5–2.5 μ long. The Schwann cytoplasm contains mitochondria (m.) and small dense granules (s.gr.). Collagen fibrils (c.) are seen next to the outer Schwann cell surfaces but do not extend into the gaps between the nodal processes. The myelin lamellae peel off from the inside, and regularly spaced inner mesaxons are seen in longitudinal sections. The outer mesaxon is seen only in transverse sections in this region.

given above. A complexing between the longest phospholipid molecules and cholesterol (figs. 9a, 9b) has been proposed in order to account for some of the structural properties and features of the chemical composition (Finean, 1953a, 1953b). The phospholipid is suggested to be associated with the protein component through its phosphate group, and, to facilitate this, it is held in a curled configuration through association with a cholesterol molecule (fig. 3). The curled form of the phospholipid molecule in the normal structure provides a possible explanation of the expansion observed when the structure is modified. The cholesterol is suggested to associate with the protein only indirectly through the phospholipid molecule, which may account for the relative ease with which it can be removed from the structure. It should also be noted that the cholesterol is suggested to be associated through its hydroxyl group—which in myelin is always found to be in the unesterified state. Although the lipid chemistry of myelin extends further than this, there are insufficient structural data to warrant further speculation on lipid organization, though in the structural diagram a cerebroside molecule is added to two phospholipid–cholesterol complexes to account for the greater part of the nerve lipid in a chemical sense. This is not intended as a structural unit. The phospholipid molecules available in myelin are mainly sphingomyelin and phosphatidyl serine, either of which might form the type of phospholipid–cholesterol complex suggested, but there are as yet no clear indications of the role of other phospholipids such as acetal phospholipid and inositol phosphatide, both of which have been indicated as myelin components. Nor are there any precise predictions as to the significance of the isolation of complex components such as gangliosides, strandin, and proteolipids. It seems feasible that, considering that all the molecules are organized in fairly compact array in the myelin structure, some of the associations may be strong enough to persist through the extraction procedure and be isolated during the subsequent fractionation. On the other hand, there is also the possibility of the extraction conditions' facilitating new associations which were not possible in the original structure, and the significance of the observations must be considered with caution.

A feature of the lipid components which has not yet been studied in detail is the degree of unsaturation among the myelin lipids, and the distribution of such unsaturations. Such information should now be obtainable through the application of vapour phase chromatography and would be of great value in assessing the possible interactions of osmium tetroxide with myelin in preparations for electron microscopy.

There is little doubt that myelin does contain a protein

FIG. 9. DRAWINGS OF PHOSPHOLIPID AND CHOLESTEROL MOLECULES



a b

a: molecular model of a phospholipid molecule (1:2-diacyl phosphatidylethanolamine); views in two mutually perpendicular directions

b: structural formula of cholesterol together with the approximate outline of the corresponding molecular model

component but as yet there is little reliable information concerning its nature. It is predicted from diffraction and electron-microscope data to be a double layer of protein about 30 Å thick originating in the apposition of two surface monolayers of protein from the original Schwann cell. It may be the very stable protein component, neurokeratin, which resists lipid solvents and enzymic digestion (Ewald & Kühne, 1874–77). The proteolipid protein has a somewhat similar composition (LeBaron & Folch, 1956), but it is difficult to establish that either has any direct connexion with the lamellar protein of the myelin sheath.

The amount and disposition of water in myelin is also somewhat uncertain. It is suggested that the water content of myelin is about 50% (Finean, 1957b), though values as low as 30% (Schmitt *et al.* 1941) and as high as 65% (Engstrom & Luthy, 1950; Shanes & Berman, 1955) have been proposed from various studies of the problem. *x*-Ray diffraction studies show that the water held between the layers must be largely organized in relation to the hydrophilic groups of the lipid and protein components, and that it is essential for the stability of some of the lipid–protein associations (Finean, 1957b). The effects of the removal of such water components are again of great importance in relation to the interpretation of electron micrographs.

There is at the moment no single direct approach to the determination of the detailed molecular organization of myelin, but the combined application of electron microscopy, *x*-ray diffraction, and chemical analysis is gradually providing vital clues concerning the most probable relationships between lipid, protein, and water components in this system.

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Notes on Contributors

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DR B. BRONTE-STEWART has recently returned to Cape Town where he is Lecturer in Medicine at the University and Physician to Groote Schuur Hospital. Formerly, he was First Assistant to the Regius Professor of Medicine at Oxford, and before that Research Associate at the University of Minnesota. In 1953, as the Cecil John Adams Travelling Fellow, he worked at St Mary's Hospital, London, and he has participated in metabolic and epidemiological studies in ischaemic heart disease in Japan, Hawaii, USA, Italy and South Africa. He has been a WHO consultant on pellagra in Basutoland and has studied the primitive Bushmen of South-West Africa. In 1956, Dr Bronte-Stewart won a Ciba Foundation award for research on ageing, and in 1951 was awarded the Joseph Arenow Prize for medical research. Past publications include: "The anaemia of adult scurvy" (*Quart. J. Med.* 1953, **22**, 309); with R. H. Heptinstall, "The relationship between experimental hypertension and cholesterol-induced atheroma in rabbits" (*J. Path. Bact.* 1954, **68**, 407); with A. Antonis, L. Eales & J. F. Brock, "Effects of feeding different fats on serum-cholesterol level" (*Lancet*, 1956, **1**, 521).

DR J. W. CORNFORTH has been a member since 1946 of the Scientific Staff of the Medical Research Council, at the National Institute for Medical Research, London. He contributed to the chemistry of penicillin and was associated with Sir Robert Robinson in the total synthesis of steroids. His work, published chiefly in the *Journal of the Chemical Society* and the *Biochemical Journal*, ranges from pure organic chemistry to experiments on biosynthesis. He is a Corday-Morgan Medallist of the Chemical Society and a Fellow of the Royal Society.

DR J. B. FINEAN is Lecturer in the Department of Medical Biochemistry and Pharmacology, University of Birmingham. After several years' research work at the Davy-Faraday Research Laboratory of the Royal Institution, London, he visited the Department of Biology, Massachusetts Institute of Technology, USA, as a Rockefeller Fellow, and then returned to Birmingham. He has collaborated in studies on bone ultrastructure at the Karolinska Institutet in Stockholm, and in 1957 he was for several months Visiting Professor at the Venezuelan Institute for Neurology (IVNIC) in Caracas, Venezuela. His chief research interests are in the molecular structures of lipid and lipoprotein systems, particularly of the nerve myelin sheath (numerous publications in *Experimental Cell Research*, *Biochimica et Biophysica Acta*, *Journal of Biophysical and Biochemical Cytology*, and elsewhere). He has recently published a book entitled *Biological ultrastructure*, 1958), in collaboration with Professor Arne Engström.

DR S. J. FOLLEY is Head of the Physiology Department of the National Institute for Research in Dairying, Shinfield, Reading, with the rank of Deputy Chief Scientific Officer. He contributed a paper on hormones in mammary growth and function to a previous number of the Bulletin (*Brit. med. Bull.* 1955, **11**, 145), and three papers on the physiology of the mammary gland to a still earlier number (*Brit. med. Bull.* 1947, **5**, 130, 135 and 142); with these a biographical note was published. Dr Folley was a Visiting Professor at the Collège de France in 1953, and was elected a Fellow of the Royal Society in 1951. Since then he has written a short book entitled *The physiology and biochemistry of lactation*, which was published in 1956.

PROFESSOR A. C. FRAZER has held the Chair of Medical Biochemistry and Pharmacology, University of Birmingham, since 1943; from 1932 to 1942, he was Lecturer (Acting Professor 1934–35) in Physiology at St Mary's Hospital Medical School. His work is concerned with particulate fat, blood lipids, and the properties of oil–water interfaces; with normal and abnormal fat absorption, and the physiology of the small intestine in man and animals; and with food research and toxicology. His work has been published in some 150 scientific papers in various journals. Some of these publications, which cover his main fields of work, are: studies on the effects of treatment with chlorine dioxide on the properties of wheat flour, written in collaboration with other investigators (*J. Sci. Fd. Agric.* 1956, **7**, 361, 371, 375, 464); "La stéatorrhée" (*Expos. ann. Biochim. méd.* 1957, **19**, 87; and a chapter on some surface phenomena in the study of the absorption of fat from the small intestine in the forthcoming volume, *Surface phenomena in chemistry and biology*, edited by J. F. Danielli, K. G. A. Pankhurst & A. C. Riddiford, to be published in 1958. Professor Frazer was awarded the Buckston Browne Prize by the Harveian Society of London in 1944; and, among many other lectures, delivered the Oliver-Sharpey Lecture, Royal College of Physicians of London, in 1956. He became an honorary foreign member of the Société Belge de Gastro-entérologie and of the Société Philomathique de Paris in 1948, and an honorary foreign member of the Royal Flemish Academy of Sciences, Letters and Fine Arts of Belgium in 1953.

DR J. E. FRENCH is a university demonstrator at the Sir William Dunn School of Pathology, Oxford. He was formerly a member of the Department of Pathology, Radcliffe Infirmary, Oxford, and in 1951 he spent a year in the USA as a Rockefeller Travelling Fellow at the University of Chicago. His main research interest is in the pathogenesis of coronary thrombosis and atherosclerosis and he has contributed chapters on these subjects to *General pathology*, edited by H. W. Florey (2nd ed., 1958). His experimental work on fat transport on which he writes in this number of the Bulletin was undertaken in view of the association between disorders of lipid transport and coronary disease.

DR J. GLOVER was appointed Leverhulme Lecturer in Biochemistry at the University of Liverpool in 1951 where, with the assistance of a number of research students, he is carrying out work on the metabolism of carotenoids, vitamin A and sterols, using ^{14}C-labelled compounds prepared biosynthetically. He graduated from the Honours School of Chemistry at the Queen's University, Belfast, in 1940 and carried out research in the same Department, obtaining the M.Sc. degree in 1941. In October 1941 he was appointed Experimental Officer in the Chemical Inspection Department, Ministry of Supply, where he was engaged on analytical problems involving physico-chemical methods. In October 1945, with a Musgrave Research Fellowship (from The Queen's University, Belfast), Dr Glover commenced research on the biochemistry of vitamin A with Professor R. A. Morton, F.R.S., at Liverpool University, and obtained the Ph.D. degree. He was appointed Assistant Lecturer in the Department from October 1947 and continued research on vitamin A and provitamin D. In 1949, he was awarded a Commonwealth Fund Fellowship to study—in the USA—the use of radioisotopes in biochemical problems. Working with Professor M. D. Kamen at Washington University, St Louis, Dr Glover studied the nature of the first compounds formed when $^{14}CO_2$ and [2-^{14}C]acetate are fixed by photosynthetic bacteria. Three main publications covering different phases of the work are: with T. W. Goodwin & R. A. Morton, "Conversion of β-carotene into vitamin A in the intestine of the rat" (*Biochem. J.* 1948, **43**, 512); chapters in *Modern methods of plant analysis* (vol. I, 1956, edited by K. Paech & M. V. Tracey); and with C. Green, "The distribution and transport of sterols across the intestinal mucosa of the guinea pig" (*Biochem. J.* 1957, **67**, 308).

DR PRISCILLA HELE studied medicine at Cambridge, and at University College Hospital, London. After serving in a junior house appointment, she returned to Cambridge, where she completed a Ph.D. in biochemistry. From 1950 to 1953 she held postdoctoral fellowships at the University of Wisconsin with Dr David E. Green's unit, at the Institute for Enzyme Research. She is now a member of the Scientific Staff of the Medical Research Council at the Experimental Radiopathology Research Unit, Hammersmith Hospital. She has published work (either singly or in collaboration) upon the metabolism of the intestinal mucosa, upon various enzyme systems utilizing coenzyme A, and (in collaboration with Dr G. Popják) upon fatty acid synthesis. She is at present studying certain aspects of amino-acid activation.

DR A. T. JAMES has, since 1950, been a member of the Scientific Staff of the National Institute for Medical Research, London. He contributed a paper on gas–liquid chromatography, in collaboration with Dr A. J. P. Martin, to a number of the Bulletin on chromatography (*Brit. med. Bull.* 1954, **10**, 170), with which a biographical note was published. Since 1956 Dr James has been developing gas chromatography for the separation, identification and quantitative determination of the long-chain fatty acids. The technique has been used to study various clinical and biochemical problems in fat metabolism.

DR J. E. LOVELOCK, on leaving Manchester University, where he received a degree in chemistry, joined the staff of the National Institute for Medical Research, London, in 1941, and in 1946 was appointed to the permanent Scientific Staff of the Medical Research Council. Papers by Dr Lovelock have been published on the following subjects: the transmission of respiratory tract infections; blood coagulation; the effects of freezing on living cell tissue and on whole animals; the mode of action of certain detergents in the chemotherapy of experimental tuberculosis; and the design of scientific instruments. His present work concerns the structure and biosynthetic activity of the human red blood cell; and the lipid composition of the blood in human subjects in health and

disease. Among his more important publications are: "A sensitive detector for gas chromatography" (*J. Chromatography*, 1958, **1**, 35); "The denaturation of lipid-protein complexes as a cause of damage by freezing" (*Proc. roy. Soc.* B, 1957, **147**, 427); and "The physical instability of human red blood cells and its possible importance in their senescence" (in: *Ciba Foundation Colloquia on Ageing*, 1956, **2**, 215).

DR MARY L. MCNAUGHT, a graduate of Glasgow University, worked for some years at the Hannah Dairy Research Institute, Ayr, on problems of ruminant digestion, with special reference to the synthesis of microbial protein, before taking up her present appointment in the Physiology Department of the National Institute for Research in Dairying, Shinfield, Reading, to study the effect of hormones on the metabolism of mammary tissue in vitro. Her publications include papers on both these topics.

DR BEDE MORRIS is the Senior Research Assistant at the Kanematsu Memorial Institute of Pathology, Sydney Hospital, Australia. He is a graduate of the Sydney University Veterinary School and has held appointments as the George Aitken Pastoral Research Trust Scholar and as an Australian National Health and Medical Research Council Fellow. He has worked at Sydney Hospital with Dr F. C. Courtice on problems of lipid transport. In 1956 he was awarded a Travelling Fellowship of the Australian and New Zealand Life Insurance Medical Research Fund, which he held at the Sir William Dunn School of Pathology, Oxford. He has published papers on fat transport and metabolism in the *Australian Journal of Experimental Biology and Medical Science*, the *Quarterly Journal of Experimental Physiology* and the *Journal of Physiology*.

PROFESSOR R. A. MORTON since 1944 has been Johnston Professor of Biochemistry in the University of Liverpool. He contributed a paper on carotenoids and vitamin A to an earlier number of the Bulletin (*Brit. med. Bull.* 1956, **12**, 37), in which a biographical note was published. In the course of Professor Morton's work in progress, which is concerned with the systemic mode of action of vitamin A and with derangements in sterol metabolism in various species, a new and wide-spread biocatalyst "ubiquinone" has been isolated and other new substances have been detected in unsaponifiable fractions of tissues. The biochemical roles of these compounds are being studied. His publications include numerous papers in scientific journals, and *The application of absorption spectra to the study of vitamins, hormones and coenzymes* (2nd ed., 1942). Professor Morton was a member of the Cohen Panel on Composition and Nutritive Value of Flour, which reported in 1956.

DR M. F. OLIVER is Senior Research Fellow of the Scottish Hospital Endowments Research Trust and Honorary Senior Registrar in the Department of Cardiology, Royal Infirmary, Edinburgh. Formerly he was Research Fellow (Advisory Committee for Medical Research) and Kirk Duncanson Research Fellow (Royal College of Physicians of Edinburgh), both in the same Department. For the past six years he has been concerned in clinical and experimental investigations into the aetiology of coronary disease, and its relationship to a disturbance of lipid metabolism and of endocrine function. Among his publications are the following, all in collaboration with G. S. Boyd: "The plasma lipids in coronary artery disease" (*Brit. Heart J.* 1953, **15**, 387); "The influence of the sex hormones on the circulating lipids and lipoproteins in coronary sclerosis" (*Circulation*, 1956, **13**, 82); "Endocrine aspects of coronary sclerosis" (*Lancet*, 1956, **2**, 1273); and "Hormonal aspects of coronary artery disease" (*Vitam. and Horm.* 1958, **16**, 147). Dr Oliver is a member of the British Cardiac Society, of the Medical Research Society, and of other medical societies.

DR J. C. F. POOLE is a Locke Research Fellow of the Royal Society and departmental demonstrator at the Sir William Dunn School of Pathology, University of Oxford. From 1949 to 1953 he worked in the Pathology Department, Radcliffe Infirmary, Oxford, where he studied the erythrocyte sedimentation rate in anaemia and the antibiotic sensitivity of the genus *Proteus*, worked on the development of an automatic red-cell counter and took part in the discovery of Christmas disease. He has been at the Sir William Dunn School of Pathology since 1954 where he has investigated the participation of various fatty substances in blood coagulation and, in collaboration with Professor Sir Howard Florey, the behaviour of aortic endothelium in various experimental conditions. His published work includes: "A haemorrhagic state resembling haemophilia" (*Lancet*, 1953, **1**, 122); "Antibiotic sensitivity of the genus *Proteus*" (*J. clin. Path.* 1954, **7**, 315); and "The clearing reaction and blood coagulation" (*The blood lipids and the clearing factor* (*Proceedings of IIIrd International Conference held in Brussels 26th to 28th of July 1956, on the Biochemical Problems of Lipids*), p. 379).

DR G. POPJÁK was Scientific Editor of a previous number of the Bulletin, "Isotopes in Medicine" (*Brit. med. Bull.* 1952, vol. 8, No. 2–3), in which a biographical note appeared. Since 1953 he has been Director of the Experimental Radiopathology Research Unit of the Medical Research Council at Hammersmith Hospital, London. Dr Popják is also Senior Honorary Lecturer at the Postgraduate Medical School of London and Honorary Consultant to Hammersmith Hospital. He is a foreign member of the Royal Flemish Academy of Sciences, Letters and Fine Arts of Belgium. He and Dr J. W. Cornforth have collaborated for several years in studying the biosynthesis of cholesterol. His publications are mainly on topics of lipid biosynthesis and on the application of isotopic tracers in biochemical research (e.g., *Chemistry, biochemistry and isotopic tracer technique*, Royal Institute of Chemistry, Lectures, Monographs and Reports, 1955, No. 2).

DR JAMES DAVID ROBERTSON is an Honorary Research Associate in Anatomy at University College, London. He received his B.S. degree (Honours) at the University of Alabama in 1942 and his M.D. degree from Harvard Medical School in 1945. After an internship in internal medicine and pathology at the Boston City Hospital, he served as a US naval medical officer for two years. He then received graduate training in biochemistry at the Massachusetts Institute of Technology, receiving a Ph.D. degree in 1952. After three years as Assistant Professor of Pathology and Oncology at the University of Kansas, he came to University College, London, to set up an electron-microscope laboratory in the Department of Anatomy. His principal interest is in tissue ultrastructure, particularly with reference to the nervous system, including synapses, peripheral nerve endings, nerve myelin and cell membranes. He has contributed papers to the *Proceedings of the Society for Experimental Biology and Medicine*, *Experimental Cell Research* and the *Journal of Biophysical and Biochemical Cytology*. He is a member of the Electron Microscope Society of America, Inc.; The American Association of Anatomists; The International Academy of Pathology; the Anatomical Society of Great Britain and Ireland; the Physiological Society.

DR D. S. ROBINSON is a member of the external staff of the Medical Research Council and works in the School of Pathology, Oxford, as one of a group, headed by Professor Sir Howard Florey, which is investigating the mechanism of transport of lipid in the body. Earlier work on some biochemical changes in developing muscle was carried out in Cambridge under Dr K. Bailey. In 1951 he went to the USA as a Fellow of the United States Public Health Service and worked on the specificity of certain peptidases. In Oxford he has been interested in the heparin-clearing reaction and lipid transport and in the role of ethanolamine phosphatide in blood coagulation. He has published several papers on these subjects in the *Quarterly Journal of Experimental Physiology*, including a review article on the heparin-clearing reaction (1957, **42**, 151), in collaboration with Dr J. E. French. From 1955 to 1957 Dr Robinson was the Johnston, Lawrence and Moseley Research Fellow of the Royal Society.

DR H. M. SINCLAIR is Fellow and Lecturer in Physiology and Biochemistry of Magdalen College, Oxford, and Reader in Human Nutrition in the University. He contributed two papers, "Vitamins and the nervous system" and "Vitamins and the skin" to an earlier number of the Bulletin (*Brit. med. Bull.* 1956, **12**, 18, 24), in which a biographical note was published.

Book Reviews

INTERNATIONAL CONFERENCES ON BIOCHEMICAL PROBLEMS OF LIPIDS

These Conferences were initiated by Professor R. Ruyssen of Ghent in 1953. Since then four conferences have been held, the proceedings of each having been published. The papers presented at the first conference (held at the Paleis der Academiën in June 1953) were published by the Royal Flemish Academy of Belgium: *International conference on biological problems of lipids*, Koninklijke Vlaamse Academie voor Wetenschappen, Letteren en Schone Kunsten van België, 1953) and those read at the second conference (University of Ghent, July 1955) by Butterworths Scientific Publications, *Biochemical problems of lipids*, in 1956. Reviews of the third and fourth conferences appear below. It has been the endeavour of the organizers of these conferences to restrict the discussions at any one meeting to a narrow field of the study of lipid biochemistry; this is an aim very difficult to achieve at an international conference and the volumes are consequently not very homogeneous. In spite of—or perhaps because of—this circumstance the four volumes so far published contain accounts of all the major advances of recent years in lipid biochemistry.

G. P.

The Blood Lipids and the Clearing Factor: IIIrd International Conference on Biochemical Problems of Lipids

Brussels: Koninklijke Vlaamse Academie voor Wetenschappen, Letteren en Schone Kunsten van België, 1957. 418 pages; figures. 26 × 18 cm. £3 12s. 6d.

This book contains a collection of papers on lipid metabolism and related subjects, which were presented at the Third International Conference on Biochemical Problems of Lipids, held in Belgium in July 1956. The papers are reproduced in English, French or German. The volume contains 38 articles, many by well-known European and American investigators, and is intended almost solely for the research worker in this field.

Not all the communications are concerned with the blood lipids or the clearing factor, consequently the book might have been left with the title, *Proceedings of the Third International Conference on Biochemical Problems of Lipids*. In a brief review such as this it is possible to make only a purely arbitrary selection of a few of the papers which appear to the reviewer to represent a cross-section of the subject-matter of the colloquium.

Tayeau's presentation is a somewhat historical and theoretical generalization on the constitution and stability of lipoproteins, but does not appear to contain any new data. James & Lovelock's contribution was on the fatty acid composition of blood lipids of various species. Using gas–liquid chromatographic methods, the red blood cell fatty acids of man, rat, rabbit and ox were contrasted. A comparison was made of fatty acid composition of plasma of people thought to be "atherosclerotic" against plasma from so-called "normal subjects".

Carlson presented data on the results of a separation of the serum lipoproteins by chromatography on glass powder columns.

Jobst & Schettler's contribution was on the physical and chemical changes of the blood lipids and lipoproteins in normal and lipaemic states. Lindgren, Freeman, Nichols & Gofman presented a paper on ultracentrifugal and chemical analyses of plasma, in which they outline their interpretation of these data in terms of lipoprotein structure and the role of the clearing factor in lipoprotein transformations.

Robinson, French, Harris, Morris and Poole presented a series of papers covering the interaction of chyle and plasma, the physiological removal of chylomicrons from blood, the exchange of lipids between plasma and lymph, and the relationship between the clearing reaction and the coagulation mechanism. Bragdon's presentation is a lucid account of the influence of dietary carbohydrate on the plasma lipoproteins in the rat.

The book contains many other interesting articles, and as some papers are of the nature of reviews much of the material has appeared elsewhere. Most of the essays are well documented, and each is followed by a discussion of the paper. The book would have lost little by more severe editing of this informal discussion, but nevertheless the collection of papers will prove useful to researchers in this field.

G. S. Boyd

Essential Fatty Acids: Fourth International Conference on Biochemical Problems of Lipids

London: Butterworths Scientific Publications, 1958. 282 + 4 index pages; figures. 26 × 18 cm. £2 10s.

This volume brings together the papers read at the Fourth International Conference on Biochemical Problems of Lipids, held in Oxford in July of last year. Though the brief title indicates that the main topic of the Conference was the consideration of the biochemistry of poly-unsaturated fatty acids, it is misleading in that many other aspects of lipid biochemistry were also discussed. Two sessions were devoted to "Chemical Aspects" and these included descriptions and reviews of current analytical procedures, namely, gas–liquid chromatography of poly-unsaturated fatty acids (James & Webb), alkaline isomerization, paper chromatography and near infra-red absorption (Holman, Hayes & Edmondson), the determination of *cis,cis*-linoleic acid in the presence of its geometric isomers (van Beers, Sparreboom & Keppler), the use of lipoxidase for the quantitative determination of poly-unsaturated acids containing the *cis,cis*-1,4-diene structure (MacGee, Mattson & Beck), and the isolation of phospholipids by dialysis through a rubber membrane (van Beers, de Iongh & Boldingh). Other papers presented at these two sessions described such diverse subjects as the mechanism of the oxidation of linoleic acid, with particular reference to metal catalysis (Uri), naturally occurring hydroxy- and epoxy-acids (Gunstone), the fatty acids of acetalphospholipids (Debuch), the composition of South African pilchard oil (Sutton), the polyenoic acids of fish oils (Klenk), the fatty acid composition of different Swedish diets (Bergqvist & Holmberg), biological interconversions of unsaturated acids (Mead & Howton) and the biosynthesis of fatty acids by human erythrocytes (James, Lovelock & Webb).

A session devoted to "Absorption and Distribution" comprised accounts of the intestinal absorption of lipids in frogs (Dominas, Karolczyk & Niemierko), the fate of dietary unsaturated fatty acids in ruminants (Lough & Garton) and a study of intestinal and faecal phospholipids in rats (Clément & Raulin), together with communications dealing with the effect of feeding various fats on the level of blood lipids (Eggstein & Schettler), the composition of the fatty acids of human blood serum after loading with various lipid emulsions (Pikaar, Revers & Rijsbosch) and the chromatographic separation of the cholesterol esters of human plasma (Michalec).

The sessions given over to "Biochemical Functions" received communications from several of the leading workers in the very topical field of cholesterol, diet and vascular disease; these included descriptions of the effects of dietary fats on serum cholesterol (Malmros), of the effect of diet on the serum lipids of Trappist and Benedictine monks (Groen) and of the relationship between poly-unsaturated fatty acids and circulating cholesterol (Holman & Aaes-Jørgensen). The effect of corn oil on lipid clearance in patients with ischaemic heart disease (Bronte-Stewart & Blackburn), the relationship between serum fatty acids and diet (Antonis), essential fatty acids, lipid metabolism and vascular disease (Kinsell, Michaels, Friskey & Splitter), and early atherosclerotic lesions produced experimentally in vitro (Evans, Brown & Ihrig) were described. Other papers deal with the structural function of essential fatty acids (Macmillan & Sinclair), essential fatty acid deficiency in children (Hansen, Adam, Wiese, Boelsche & Haggard), investigations on pre-albumin lipoprotein fractions of serum (Lewin, Azerad & Ghata), the metabolism in man of albumin-bound labelled fatty acids (Fredrickson & Gordon); and accounts were given of the role of fats in cell growth and respiration (Budwig), metabolic studies in fat deficiency (Panos, Finerty, Klein & Wall) and fatty acids as surface-active agents (Davies).

The discussion on each paper is included and, by way of conclusion, several contributors presented summaries relating to chemical methods, the effects of deficiency of essential fatty acids and the effects of fatty acids on serum cholesterol.

G. A. Garton

The Chemistry of the Steroids

W. Klyne. London: Methuen & Co. Ltd., 1957. 216 pages; 4 figures. 19 × 13 cm. 18s.

This little book is designed to provide ". . . an outline of the chemistry of the steroids for the non-chemical reader". Dr Klyne has kept the image of this reader steadfastly in mind, referring him to more comprehensive works for most details of structural elucidation and synthesis, but acquainting him with nearly every type of problem encountered in steroid chemistry. He must not, however, be so non-chemical that he has never tried to learn the elements of organic chemistry before he approaches this book.

There are chapters on stereochemistry, on physical properties, on the chemical properties associated with simple and complex functional groups (two chapters), on the different types of naturally occurring steroids (three chapters) and on the separation of steroids. The reader who masters this text will have a sound knowledge of the subject and should find, if he needs more detailed information, no new difficulty in understanding the original literature.

The book is well printed, the formulae are clearly drawn, and no mistakes of any kind have been noticed. The treatment of the vitamins D is somewhat out of date—the discovery of precalciferol is not too recent to have been mentioned—but for the most part every effort has been made to include the latest developments. The book can be warmly recommended as an introduction to steroid chemistry.

J. W. Cornforth

The Chemistry of Lipids of Biochemical Significance

J. A. Lovern. 2nd ed. London: Methuen & Co. Ltd., 1957. xiii + 157 pages; 2 figures. 17 × 11 cm. 12s. 6d.

This book, although entitled *The chemistry of lipids of biochemical significance*, contains just about 50% biochemistry. This is a good indication of the haziness of the border-line between the two sciences, and of the extent to which chemical structure and biological function are interrelated. The book is already in its second edition, the first having been published two years earlier; it is one of a well-known series which provides brief accounts of major fields of biochemistry for the non-specialist.

Chapter I (42 pp.) deals with the chemical structure of the many classes of lipids. Perhaps by the time the third edition appears it may be thought appropriate to include perspective formulae of some of the structures. Three-dimensional thinking in biochemistry should develop rapidly.

Chapter II (39 pp.) treats in considerable detail the preparation and analysis of lipids. The lipid chemist is for ever faced with the problem of treating mixtures of homologues and other closely related compounds in almost every group with which he deals. In no other field of chemistry dealing with natural products is this complication found to the same extent. Small wonder that methods for the preparation and analysis of lipids are tedious and often imperfect. The reviewer feels that the accounts of methods in this chapter might gain in clarity if more use were made of figures and tables.

The remaining three chapters are essentially biochemical. Chapter III (22 pp.) deals with the state of lipids in the tissues, where many of them are bound as lipoproteins or lipopolysaccharides. The nature of the many different types of binding is still very imperfectly understood.

Chapter IV (32 pp.) considers the dynamic state of lipids in the tissues. The classic work of Schoenheimer and Rittenberg on isotopic labelling of body constituents opened an entirely new approach to intermediary metabolism, and the "turnover" of lipids and their various fragments forms a fascinating and complex field for study.

Chapter V (18 pp.) completes the book with a brief discussion of the functions of the various classes of lipids. These, as the author states, are the subject of much speculation.

The book provides a sound and reliable introduction to the chemistry (and much of the biochemistry) of the lipids. The selection of references is adequate for all but specialist readers, who will turn to the larger works cited here.

W. Klyne

The Biochemistry of the Nucleic Acids

J. N. Davidson. 3rd ed. London: Methuen & Co. Ltd., 1957. viii + 248 pages; 41 figures; 4 plates. 17 × 11 cm. £1 1s.

From the time of their discovery indirect evidence pointed to two biological functions of nucleic acids: their participation in protein synthesis and their fundamental role in the genetic mechanism of the cell. However, even in 1950, when the first edition of Professor Davidson's book was published, the only direct evidence for a biological role of nucleic acids came from the bacterial transforming deoxyribonucleic acids, and the chemical structure of nucleic acids was still uncertain. In the last eight years progress in this field has been astonishingly rapid; in particular, the genetic role of nucleic acids in virus multiplication has been established and the mechanism by which nucleic acids participate in protein synthesis is beginning to be understood. At the same time, the elucidation of the general features of nucleic acid structure has provided a basis for the interpretation of genetic phenomena in chemical terms.

The need for an introductory account of the biochemistry of nucleic acids, particularly for workers in related fields, has been well demonstrated by the success of the two previous editions of this book. In the third edition the book has been extensively revised and covers the literature up to the end of 1956. An outline is given of the chemical structure and physical properties of the nucleic acids, their biosynthesis, their position in the structure of the cell and their biological function. An introduction to the analytical methods employed in nucleic acid chemistry is also provided. While a volume of this size can hardly be comprehensive, its value is greatly enhanced by a very careful selection of references. Possibly too much space is devoted to the metabolic activity and distribution of nucleic acids in tissues, compared with the relatively brief treatment of the part played by nucleic acids in bacterial transformations and in virus multiplication, from which most of the fundamental biological discoveries in this field have arisen. The paragraph on bacteriophage-mediated transduction does not describe the phenomenon generally meant by this term, and no mention is made of the lysogenic bacteria. To those unacquainted with recent developments in bacteriophage genetics the discussion on p. 238 of the size of the genetic unit in T2 bacteriophage will be somewhat misleading.

There is no doubt that this edition will be as successful as its predecessors in providing a general introduction to this field.

J. D. Smith

Chemistry of the Steroids

Charles W. Shoppee. London: Butterworths Scientific Publications, 1958. vii + 314 pages. 22 × 14 cm. £2 10s.

Too many books are too expensive nowadays for purchase by the individual. Professor C. W. Shoppee's concise volume, in contrast, is well within the purse of every scientist. Its very conciseness, however, introduces the problem of what to include and what to omit, and it is on this point alone that criticism can be made. Professor Shoppee's book is unsuitable for those with no real knowledge of steroid chemistry. It is essentially a review and summary of steroid progress during the decade 1945–55, written by the sure hand of a master. There is, consequently, no real point in retaining some of the historical material, which could well be omitted, thereby releasing space for such rapidly expanding topics as biogenesis (p. 29). Apart from this minor criticism, the book may be warmly recommended to all those interested in, and with knowledge of, this important subject. To the research worker it is a fund of information to read again and again.

Chapter I contains introductory historical material. It covers nomenclature, stereochemistry, determination of structure and biogenesis.

Chapter II deals with the sterols, bile acids and related compounds. The section dealing with the reactions of the 3-hydroxy-Δ^5-moiety is very well done and contains much information of value even to the specialist. The Cornforth & Robinson and the Woodward syntheses of steroidal structures are outlined. Ergosterol and its transformation products, the lesser-known steroids and bile acids are all discussed. Stereochemical relationships are stressed throughout.

Chapter III deals with the sex hormones. The oestrogenic hormones are well covered and their total syntheses outlined. The

section on the gestogens is excellent. Progesterone and its hydroxylated and transformation products are reviewed. Ethisterone and 19-norethisterone are mentioned. Biological properties are discussed. The statement on p. 129 that progesterone is manufactured from dehydro*epi*androsterone is not valid for Great Britain or the USA. Androgenic hormones are discussed on pp. 137–156: the more important natural androgens are reviewed and their biological properties tabulated; the 19-norsteroids and the homosteroids are lucidly treated; the biological importance of the 19-nor structure is stressed.

Chapter IV gives a concise account of the chemistry and transformations of the major adrenal steroidal products, including aldosterone. In addition the more important methods for introducing an 11-hydroxyl group and the adrenocortical side-chain are given due weight. Biological activities are referred to where desirable. There is a section on total synthesis. The employment of microbiological resolution of synthetic DL-aldosterone is mentioned.

Chapters V and VI deal with the cardiotonic glycosides and with the saponins. Such glycosides as strophanthidin, digitoxigenin and getoxigenin are discussed in some detail. The interesting 11-oxygenated sarmentogenin and 11 : 12-dioxygenated sarmentogenin group are ably collected (pp. 250–252). The constitution of the saponins, including the stereoisomerism of the spiroketal side-chain, is clearly presented and the structures of the sapogenins correlated. Their degradation to the steroid hormones is outlined on p. 291, although the technical importance of diosgenin as raw material for steroid production is insufficiently stressed.

The book is attractively bound. Ample structural formulae are given throughout the text.

V. Petrow

Cholesterol: Chemistry, Biochemistry, and Pathology

Edited by *Robert P. Cook*. New York: Academic Press Inc.; London: Academic Books Ltd., 1958. xii + 542 pages; figures. 23 × 15 cm. £5 7s. 6d.

In the now classical *Lehrbuch der organischen Chemie* by Meyer & Jacobson, published 34 years ago, one may read that "The physiological literature for decades has been extraordinarily rich in numerous data of the cholesterol content of various animal fluids and tissues . . . Yet the methods of analysis have only recently acquired the degree of accuracy to be desired, and therefore many of the older data are little to be relied upon." Again: "On the part which is played by cholesterol in the life-process . . . generally accepted opinions have not yet been achieved" (reviewer's translation).

It is matter for humility—or a measure of the difficulty of the problem—that in this new book it has to be emphasized by R. P. Cook & J. B. M. Rattray not only that determinations of cholesterol are inconsistent with each other when different methods are used, but that the "cholesterol" determined is not a single chemical compound, and further, that D. Adlersberg & H. Sobotka can still say that ". . . one does not know the significance of the increases and decreases of cholesterol in the body and its organs". Since the days of Meyer and Jacobson there has been the tremendous flood of work on the steroid hormones, with its repercussions on the chemistry of cholesterol, and the intense current interest in atherosclerosis to stimulate biochemical and clinical research. One gets the impression that we are on the threshold of a considerable advance, and this volume may well play an important part in that advance. Dr Cook has organized a most successful coverage of the field by international collaboration, and the result is neither a textbook assuming for didactic purposes that all the problems can be simplified and regarded as solved in logical fashion, nor one of those irritating reviews in which mutually irreconcilable results are juxtaposed in a breathless stream of abstracts. Accounts are given of representative work in all branches, in form varying from an extremely useful, if rather inelegant, tabulation of cholesterol chemistry by Peter Bladon to a fascinating essay on the conversion of cholesterol to steroid hormones by Oscar Hechter and a singularly brief chapter on treatment of disorders of cholesterol metabolism by Irvine H. Page. A long and important chapter on pathological manifestations of abnormal cholesterol metabolism by D. Adlersberg & H. Sobotka ends with the sober conclusion that "Present knowledge is not enough advanced to ascribe to cholesterol a primary role in atherosclerosis, but its experimental administration to animals results in pathological conditions resembling those found in human vascular disease".

It would probably be considered impertinent to suggest that every busily occupied clinician who asks for "a blood cholesterol" should dip into this book to make sure of what he is asking for and why, but, seriously speaking, these authoritative articles with the many references, among which reviews are specially indicated, constitute a volume to which those outside the field may refer to find out what is being done, whilst those active within the field may profitably read it to be sure that they have missed no information, to appreciate other points of view and to gain perspective by learning of work in other parts of a crowded subject.

R. K. Callow

Radioisotope Laboratory Techniques

R. A. Faires & B. H. Parks. London: George Newnes Ltd., 1958. xii + 244 pages; 4 plates + 89 figures. 22 × 14 cm. £1 5s.

The Harwell isotope school is now well known in many parts of the world and has all the appearances of a permanent institution. Sooner or later a manual was bound to appear based on the valuable course of instruction which is given there. It is most satisfactory that such a book has now appeared and that it should include R. A. Faires, the head of the school, as one of its two authors.

The book is essentially intended for beginners and comprises mainly the basic physics and chemistry of isotopes. It will therefore be of greatest interest to workers in other fields and especially to biologists who in any case occupy annually a substantial proportion of the school places. The emphasis throughout is on practical matters, and most benefit will be derived by those who have been precipitated into working with isotopes without an adequate training in the natural sciences.

Following a preface by Dr Henry Seligman, there are some twenty short chapters each with a brief summary under the chapter title. Typical subjects dealt with are: properties of radiations, hazard control, counting techniques, applications of isotopes; and the book concludes with six appendices of physical data in everyday use. The choice of subject-matter is excellent, as one would expect of experienced teachers, although it is obvious that the authors are more at home in some fields than in others. The treatment of counting techniques strikes the reviewer as being particularly thorough, and also the chapter on the statistics of counting. The beginner could easily select the most suitable equipment to buy for his particular problem and proceed to use it with the kind of instruction given in this book, and there must be many isotope users who want only this kind of direct instruction.

However, as so often happens with a first edition, there are numerous errors: those of spelling are of little consequence but some mistakes in equations may seriously mislead the reader. These should be put right by an erratum slip as soon as possible, and the quality of the half-tone plates should be improved. This is basically a most useful book at not too high a price.

A. S. McFarlane

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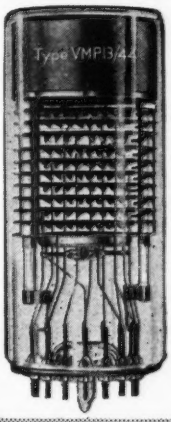

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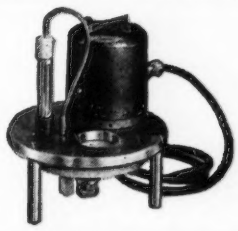

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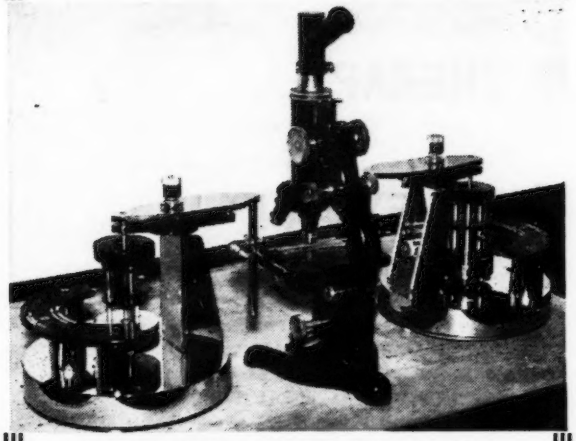

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